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14. ABSTRACT
Cancer growth and spread is dependent on new blood vessel formation, i.e. angiogenesis. A tumor mass cannot develop into a life-threatening condition without angiogenesis. Obstructing the recruitment of new blood vessels to the tumor through administration of antiangiogenic agents will hinder cancer progression. We propose the use of marrow stromal cells (MSCs) for an investigative gene discovery program to identify new genes involved in blood vessel formation. MSCs, a normal cell type from the bone marrow, can spontaneously turn into blood vessels (MSC-mediated vasculogenesis) in experimental animals. Therefore, we propose that MSCs recapitulate the ontogeny of blood vessel formation and serve to identify novel angiogenesis promoters and potential new pharmacological targets. To test this hypothesis, we will utilize a cell biology and molecular genetic experimental approach. Products thus identified as involved in MSC-mediated vasculogenesis may become new cancer "antiangiogenesis" targets for either a classic pharmacological approach or for cell and gene therapy therapeutic strategies. The utilization of antiangiogenic agents for cancer treatment holds certain advantages over chemotherapeutic drugs, such as the destruction uniquely of tumor-associated normal blood vessels and not of other normal tissue such as bone marrow. Also, unlike chemotherapy, drug resistance is not an issue with antiangiogenic compounds.

15. SUBJECT TERMS
Marrow stroma, breast cancer, angiogenesis gene therapy, gene discovery

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Final Report for Concept Award BC023590 "Mesenchymal Stem Cells for Vascular Target Discovery in Breast Cancer-Associated Angiogenesis" DAMD17-03-1-0633

INTRODUCTION

Progression and metastasis of breast cancer is dependent on neoangiogenesis (Folkman J. *Journal of the National Cancer Institute* 82:4, 1990), and host-derived mesenchymal progenitor cells contribute to this phenomenon (Hombauer H and Minguell JJ. *British Journal of Cancer* 82:1290, 2000). Marrow-derived stromal cells (MSCs) display mesenchymal plasticity, are amenable to *ex vivo* tissue culture and can serve as a robust model of mesenchymal morphogenesis. We recently demonstrated that culture-expanded MSCs can transdifferentiate into blood vessels expressing the endothelial marker CD31 *in vivo* and that they also recruit a robust, vascular endothelial growth factor (VEGF)-dependent, angiogenic response from host-derived structures (Al-Khaldi A. et al. *Gene Therapy* 10(8): 621-629, 2003). This discovery may allow us unprecedented insights in the biology of postnatal vasculogenesis and angiogenesis, especially in the setting of cancer.

RATIONALE AND OBJECTIVES

We propose that MSCs recapitulate the ontogeny of vasculogensis and that the analysis of this process will give novel insights in the molecular genetic events associated with cancer-driven angiogenesis. Gene products identified as involved in MSC-mediated vasculogenic response to cancer may then become new breast cancer "anti-angiogenesis" targets.
Specific aims are to perform gene array analysis of vascular differentiated murine MSCs in the setting of breast cancer. Both *in vitro* and *in vivo* MSC differentiation – and gene expression profiling - will be analyzed and validated.

PROPOSED METHODS

We have already shown that a mixed population of MSCs harvested from C57Bl/6 mice can be retrovirally-labelled to express the green fluorescent protein (GFP) and clonal MSC populations can be isolated and phenotypically and genotypically analyzed by flow cytometry and gene chip analysis, respectively. We have also shown that MSCs can then be stimulated to differentiate *in vitro* into an endothelial phenotype in presence of recombinant mitogens such as, VEGF 185 and basic fibroblast growth factor (bFGF). The input and output MSCs under different "controlled" *in vitro* conditions can be compared and analyzed by gene expression profiling as detailed below. Furthermore, 10^6 clonal "endothelial-null" MSC cells can be admixed in a 0.5 ml Matrigel plug and embedded in the subcutaneous space in recipient C57Bl/6 mice. As we have shown (Al-Khaldi A. et al. *Gene Therapy* 10(8): 621-629, 2003), a vasculogenic/angiogenic response will occur as early as 2 weeks post-implantation and persists for at least 4 weeks. The Matrigel implant can be easily removed 2-4 weeks post-implantation and up to 10^5 live GFP-positive MSCs retrieved by collagenase digestion of the Matrigel plug and sorted by flow cytometry. Sorting of these retrieved cells based on co-expression of the GFP marker and cell-surface endothelial markers (such as CD31) will allow purification of input MSCs [and eliminate contaminating GFP-null host-derived cells] having undergone endothelial differentiation *in vivo*. The number of MSCs thus extracted is sufficient to allow for linear RNA amplification to quantities amenable to analysis by Affymetrix-based gene chip technologies. We propose that comparing the gene expression profile of input "GFP+, endothelial-null" MSCs with output "GFP+, endothelial-

positive" MSCs will allow us to define molecular genetic events associated with post-natal vasculogenesis. The experiments here proposed can be done with MSCs alone or with MSCs admixed with cancer cell lines *in vitro* and within a Matrigel plug *in vivo*. We will thus determine whether cancer-associated angiogenic/vasculogenic responses are qualitatively distinct from vascular differentiation in the absence of tumor cells. To test this hypothesis, we will use mouse models of breast cancer. 4T1 and DA3 are murine breast cancer cell lines that generate metastatic and local breast carcinoma respectively in Balb/c mice and both these cell lines will model for breast cancer-associated vasculogenesis. MSCs derived from Balb/c mice will be admixed with these breast tumor cell lines and will be analyzed *in vitro* and *in vivo*, as described above.

KEY RESEARCH ACCOMPLISHED, REPORTABLE OUTCOMES

Although we were unable to completed several of the objectives of the approved Statement of Work (indicated here below in italics) due to unexpected technical delays, we have conducted several related experiments beneficial to the advancement of this proposal and understanding of vasculogenesis.

Task 1. In Vitro experiments, Months 1-3:

Perform gene array analysis of in vitro-induced vasculogenesis with murine marrow stromal cells (MSCs) in the presence of recombinant VEGF 185 and recombinant bFGF.

We have isolated primary murine MSCs from C57Bl/6 mice and genetically engineered them *in vitro* with retroviral particles to express the green fluorescent protein (GFP) reporter. We selected monoclonal populations of these GFP+ murine MSCs and conducted flow cytometry analysis to determine their phenotype. Specifically, we determined if these cells express the following cell surface antigens: CD31, CD34, CD44, CD45, CD117, Flk1 and Tie2. Upon noticing that all clones were CD31- and that most clones were CD34- but one was CD34+, we investigated if CD34 expression had an effect on the potential endothelial plasticity of MSCs and consequently performed the *in vivo* experiments described under Task 2 here below. Moreover, total RNA was isolated from CD34+ and CD34- MSCs and gene expression analysis was conducted (please see manuscript attached). Briefly, gene expression analysis of these recovered MSCs indicated no significant differences in the expression of VEGFs A and B, but showed that CD34+ MSCs upregulated several supplementary angiogenesis-associated genes (please see manuscript attached).

Since one of the primary instigators of tumor-associated angiogenesis is hypoxia and MSCs target to this hypoxic environment we conducted additional experiments to determine whether hypoxia influences the angiogenic phenotype of MSCs. These experiments have helped clarify the intracellular (genomic) and extracellular (proteomic) environment of murine MSCs exposed to 24-hours hypoxia. Collection of the culture media was done for proteomic analyses, and extraction total cellular RNA for performed for gene expression. Using the same culture conditions we will also cocultured (contact independent) MSCs with 4T1 cells (1:1 ratio) and isolated

the MSCs RNA after 24-hours of hypoxia or normoxia for genomic analysis (See task 3). The in vitro gene profiles were generated using the Affymetrix® Murine Genome MOE arrays, contains probe sets interrogating approximately 45,000 full-length mouse genes and EST clusters from the UniGene database. Reverse transcription, labeling and array hybridizations were performed by the microarray facility at the Ontario Genomics Innovation Centre (OGIC) (www.ottawagenomecenter.ca) within the Ottawa Health Research Institute (OHRI) (http://www.ohri.ca). Data mining and analyses will be performed by our laboratory using multiple scoring clustering methods followed by systematic classification of differentially regulated genes into functional groups allowing us to visualize the data in the context of biological pathways. Following these guidelines, 24 hours of hypoxia influenced the expression of 444 gene by at least 2.5 fold in MSCs (See Figure 1). Of these 444 genes, 173 genes were increased due to hypoxia while 271 were decreased. Of these 445 genes 179 were functionally unclassified. Functionally hypoxia up-regulated genes involved with glycoloysis, apoptosis secretion and cell mobility. In the setting of angiogenesis hypoxia significantly increased the expression of Vegfa as well as members of the TGF, and IGF families.

For proteomic analyses, we first assessed the influence on hypoxia on the angiogenic potential of MSCs using an angiogenesis antibody array from Panomics. This array can detect picogram levels of 19 angiogenesis-related cytokines allowing us an unbiased view of how hypoxia influences the secretion of angiogenic mediators from MSCs. Based upon these analyses we found that MSCs basally secrete detectable amounts of the pro-angiogenic factors VEGF, IL-6, as well as, the anti-angiogenic factors TIMP-1 and TIMP-2. Twenty-four hours of hypoxia appears to enhance the secretion of VEGF from MSCs, however the factor which is most dramatically altered by hypoxia was leptin (See Figure 2). Leptin is a well characterized pro-angiogenic factor *(Cao R,Proc Natl Acad Sci U S A. 2001 May 22;98(11):6390-5.)* and in the setting of breast-cancer high levels of the leptin receptor are found on mammary carcinoma cells (*Ishikawa M, Clin Cancer Res. 2004 Jul 1;10(13):4325-31.*). In subsequent experiments we will test the hypothesis that MSCs migrating into the hypoxic environment of a tumor secrete leptin and Vegf, which synergistically enhance tumor-associated angiogenesis.

Since our angiogenesis antibody array only allowed us to screen well known mediators of angiogenesis we performed an additional in vitro proteomic screen using isotope-code affinity tag (ICAT) and mass-spectrometry peptide mass fingerprinting technologies. This technique has the potential to identify thousands of proteins in a single experiment and is sensitive into the fetogram range. ICAT-labeling and mass-spectrometry experimental procedures were performed at the University of Louisville Core Proteomics Laboratory (www.proteomelab.org) through collaboration with Dr. Eugenia Wang. Technical difficulties have precluded a comprehensive list of proteins secreted from MSCs, however data from multiple experiments indicate that PAI-1 secretion is increased 1.5X when MSCs are exposed to a hypoxic environment. Subsequent analyses using western blotting have confirmed this observation (See Figure 3). Based upon this data we have generated MSCs

from PAI-1 knock-out mice and will assess the angiogenic phenotype of these MSCs in subsequent analyses.

Task 2. In Vivo experiments without tumor cells, Months 4-6:

Perform gene array analysis of in vivo-induced vasculogenesis with Matrigel-embedded murine MSCs in the absence of tumor cells in Balb/c mice.

Although not yet conducted with MSCs derived from Balb/c mice, we did however perform related *in vivo* experiments with MSCs derived from C57Bl/6 mice. More specifically, we selected from the above-mentioned GFP gene-modified MSC populations, one that was CD34+ and one CD34- and utilized these in our vasculogenic assay, whereby 4 million MSCs were admixed with a typeIV collagen-based matrix Matrigel^{TM} in a final volume of 0.5ml. This Matrigel-MSC mixture was then injected subcutaneously in syngeneic C57Bl/6 mice where it formed a semi-solid implant which was surgically removed 15 days later and collagenase digested to recover the cells. Flow cytometry analysis was then conducted on the GFP+ MSCs and revealed that 2-5 % of these MSCs had transdifferentiated from CD31- to CD31+ cells and that the MSCs that were CD34+ appeared more prone to becoming CD31+ and to recruiting hematopoietic cells from the host animal. Gene expression analysis of these recovered MSCs has not yet been carried out due to technical obstacles in obtain sufficient high quality RNA from the implants *in vivo*.

Task 3. In Vivo experiments with breast tumor cells, Months 7-12:

Perform gene array analysis of in vivo-induced vasculogenesis with Matrigel-embedded murine MSCs in the presence of DA3 breast tumor cells (Balb/c mice).

Perform gene array analysis of in vivo-induced vasculogenesis with Matrigel-embedded murine MSCs in the presence of 4T1 breast tumor cells (Balb/c mice).

At the time of this report we had not been successful in conduction gene array analysis of Matrigel embedded murine MSCs in the presence of DA3 or 4T1 breast tumor cells. This was due to technical obstacles in obtaining sufficient high quality RNA from the implants *in vivo*. However, we have clear evidence that MSCs have the ability to accelerate tumor growth *in vivo* (See Figure 4). Based upon these observation we conducted *in vitro* experiments to determine whether tumor cells secrete factors that enhance the angiogenic potential of MSCs. Using a multiwell co-culture system we seeded both MSCs and 4T1 cells so that they could exchange soluble factors, but were not in direct contact with one another. Following serum removal these cells were cultured for 24 hours and the RNA collected from the MSCs for gene expression profiling and compared to the gene profile of MSCs without exposure to 4T1 paracrine factors (See Figure 5). Microarray analysis revealed that MSCs in the presence of 4T1 cells have a phenotype that is far more angiogenic. Specifically co-culture of MSCs with 4T1 enhanced the

MSC mRNA expression of Vegfa and increased the expression several other angiogenesis mediators including members of the angiogpoietin/ephrin, chemokine/interleukin, matrix metalloproteinases, IGF and PDGF families. In addition the mRNA of several regulators of eicosanoid synthesis were also increased in MSCs when they were co-cultured with 4T1 breast cancer cells. In subsequent experiments we plan to confirm our Affymetrix observations with real-time PCR. Furthermore we will use our proteomic techiniques to identify the factor(s) which 4T1 cells the secrete to enhance the angiogenic potential of MSCs.

We have prepared a manuscript on the findings summarized above related to the effect of CD34 cell surface antigen expression on the *in vivo* ability of MSCs to differentiate into endothelial cells. The manuscript entitled "CD34 expression by murine marrow stromal cells and neovascularization" was submitted to *Stem Cells* in July 2005 (See attached manuscript). Furthermore we are currently confirming the changes in gene/protein expression we found from MSC exposed to either hypoxia or tumor cells with real-time PCR and western blotting. In the future we hope to optimize our methodology for assessing secreted protein expression with Mass Spectrometry and will begin assessing the permissive/submissive effects of angiogenic targets on breast-cancer tumor growth *in vivo*.

CONCLUSION

Even though we have not completed all of the objectives of the approved Statement of Work due to unforeseen technical difficulties, we have performed related experiments for the advancement of this proposal and understanding of vasculogenesis. Specifically the data we genereated provided a spectrum of genetic, biochemical and physiological mechanisms underlying cancer-associate angiogenesis. Specifically we have identified three types of "angiomic" events in these studies: (i) de novo up-regulation of angiogenic markers in the absence of cancer, i.e: "normal angiogenesis", (ii) up-regulation of angiogenic markers in the presence of breast cancer cell lines, i.e: "cancer-associated angiogenesis". (iii) Novel synergistic up-regulation of angiogenic markers when MSCs are present with breast cancer cell lines, i.e. "hypoxia stimulated cancer-associated angiogenesis". Overall theses analyses have identified several proteins which may be intimately involved in cancer-associated MSC-driven vasculogenesis and thus has help generate a database for development of novel anti-angiogenic pharmaceuticals.

Appendix 1

Appendix 1

CD34 Expression by Murine Marrow Stromal Cells and Neovascularization

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Abstract

Anchorage-dependent Marrow Stromal Cells (MSCs) induce neovascularization in vivo. It has been observed that CD34 expression in a mixed population of mouse MSCs is variegated. Since CD34 identifies hemangiopoietic precursors, we tested whether its expression could serve as a surrogate marker of MSCs possessing neovascularization potential.

A mixed population of MSCs derived from C57Bl/6 mice transduced to express the green fluorescent protein (GFP) from which pure MSC/CD34+ and MSC/CD34^{null} clones were selected, admixed with Matrigel™, and injected subcutaneously into isogenic mice. Implants were excised at 15 days, dissociated, and the single cell preparation submitted to flow cytometric analysis. GFP-expressing MSC/CD34+ were analyzed for the acquired expression of the CD31/PECAM-1 endothelial marker and found to have significantly more CD31 expression compared to MSC/CD34^{null} cells (10.7 ± 8.4% vs. 3.1 ± 0.6%; $p<0.05$). A significantly greater proportion of host-derived endothelial cells (ECs) (CD31+/CD45-) were also recruited in the MSC/CD34+ implants. Immunohistochemistry of the implants confirmed the neovascularization advantage of MSC/CD34+ over MSC/CD34^{null} cells based on blood vessel density (BVD). Gene expression profiling revealed no significant differences in the expression of VEGFs A and B. However, CD34+ MSCs upregulated a number of supplementary angiogenesis associated genes.

CD34+ and CD34^{null} MSCs have the ability to initiate post-natal vasculogenesis. CD34+ cells are more efficient at recruiting host-derived ECs

and differentiating into CD31+ cells in vivo suggesting that CD34 expression is not a mandatory surrogate marker of angiogenic plasticity of MSCs, but is associated with a more robust host-derived vascular response.

Key Words: Angiogenesis, vasculogenesis, bone marrow stromal cells, CD34

Introduction

It has been established that blood, marrow and cord blood derived endothelial progenitor cells (EPCs) may participate in post-natal vasculogenesis. These circulating cells of vasculogenic potential have been defined(1;2) as expressing vascular endothelial growth factor receptor 2 (VEGF-R2/Flk1) and the cell-surface sialomucin CD34, a widely utilized surrogate marker of hematopoietic stem cells(3).

We have also described that marrow-derived, anchorage-dependent, murine marrow-derived stromal cells (MSCs) display vascular plasticity in an *in vivo* Matrigel plug angiogenesis assay and have the ability to recruit a mostly host-derived angiogenic response.(4) However, in distinction to EPCs and mesenchymal adult progenitor cells (MAPCs), there is marked heterogeneity in the expression of CD34 by cultured murine MSCs *in vitro*(5). Since CD34 is widely utilized as a surrogate marker of progenitor and stem cells with potential vascular plasticity(6-9), we investigated whether MSC CD34 expression also predicted for prospective vasculogenic properties.

Materials and Methods

1. Harvest and culture of murine MSCs

Female C57Bl/6 mice were purchased from Charles River Laboratory (Laprairie Company, Quebec) from which bone marrow was harvested as previously described by us.(4) In brief, mice were sacrificed by CO_2 inhalation. Femoral and tibial bone marrow plugs were hydrostatically expelled and cells were plated on tissue culture dishes in growth media [Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% decomplemented fetal bovine serum and antibiotics (50U/ml Penicillin G and 50μg/ml Streptomycin from Wisent Inc.)] and incubated at 37°C in room air with 5% CO_2. To eliminate the hematopoietic portion of cells, the non-adherent portion was discarded five days later and media was replaced once per week. Anchorage-dependent MSCs were passaged 1:2 when the cell confluency became 80% to 90%. MSCs were cultured for ~15 passages. Animals were handled under the guidelines promulgated by the Canadian Council on Animal Care and with the Animal Welfare Act Regulations and other Federal statutes relating to animals and experiments involving animals, and adhere to the principles set forth in the Guide for Care and Use of Laboratory Animals, U.S. National Research Council, 1996.

2. Marrow Stromal Cell retroviral labeling and clonal selection

Culture expanded MSCs were retrovirally labeled to express the green fluorescent protein (GFP) reporter as previously described by us.(40) In brief, MSCs were transduced with retroparticles from ecotropic GP+E86 producers

encoding the AP2 retrovector construct(41) in the presence of $6\mu g/ml$ lipofectamine reagent (Invitrogen/Life Technologies). Following transduction, 100% of MSCs expressed the GFP reporter stably over time (data not shown). It has been previously shown that MSC clonal subsets with varying phenotypes will form when MSCs are plated at a low density.(42) We isolated MSC clonal populations by a similar limiting dilution technique. Following retroviral GFP labeling, polyclonal MSC's were plated at a low density (approximately 5-10 cells per cm^2). As visible colonies formed, they were collected with the use of filter disks immersed in trypsin 0.05%. After several minutes of incubation, the disks were removed and placed on individual culture plates with media. Twenty clonal MSC subsets were subsequently culture-expanded in the same manner as the mixed populations. Two clones: MSC/CD34+ and MSC/CD34- were subsequently characterized *in vivo*.

3. Characterization of MSC phenotype – Flow cytometric analysis

Flow cytometric analysis was used to characterize the phenotype of the MSCs (both mixed populations and clonal subsets). Immunostainings were performed by incubating 1 x 10^6 cells for 30 min at +4°C with the following monoclonal antibodies: biotinylated anti-mouse Flt4 (VEGFR-3, clone AFL4), Tie-2 (clone TEK4) from ebioscience (San Diego, CA), CD31(clone 290), CD34 (clone RAM34) revealed with streptavidin-CyCr and PE labeled anti-mouse CD13 (clone R3-242), CD44 (clone IM7), CD45 (clone 30-F11), CD117 (clone 2B8) and Flk1(VEGFR-2, clone Avas12□1) from BD biosciences (Mississauga, ON,

Canada). Cells were acquired and analyzed with CellQuest Pro software on a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA).

4. *in vivo* Matrigel angiogenesis assay

We have previously described the subcutaneous implantation of matrix-embedded MSCs admixed in Matrigel™ (Becton Dickinson, Mississauga, Ontario, Canada) to ascertain their vasculogenic and angiogenic properties *in vivo*.(4) In brief, MSCs are admixed in chilled liquid Matrigel, syringe loaded and injected subcutaneously where it forms a semi-solid plug. The plug can be surgically retrieved at latter time points and subjected to histological analysis as well as protease dissociation to retrieve populating cells for FACS analysis.

We used 5 animals to test each described clonal MSC population (MSC/CD34$^+$ and MSC/CD34$^-$). We also utilized embryonic fibroblast derived from C57Bl/6 (n=5) and cell-free Matrigel (n=5) as controls. At the time of implantation, 8x10^6 cells were resuspended in 1 ml of Matrigel™, in liquid form at 4°C. A volume of 0.5 ml of this cell/Matrigel mixture (4 million cells) was injected subcutaneously in the right flank in each test C57Bl/6 mouse. Fifteen days following implantation, mice were sacrificed and Matrigel plugs were excised for analysis. With gentle dissection, the Matrigel™ plug was removed, taking care to avoid puncturing or dividing the Matrigel™. Four implants from each group were reserved for FACS analysis of phenotype while 1 implant from each group was reserved for immunohistochemistry.

Live cells were retrieved from Matrigel plugs utilizing a protocol adapted from Salomon *and al.*(43) Briefly, retrieved implants were cut into small fragments in a solution of PBS 1X supplemented with 1.6 mg/ml type IV collagenase and 200 µg/ml DNase I (Sigma-Aldrich, Oakville, Canada,) at 37°C for 30 min. Cells were dissociated by repeated pipetting, reincubated at 37°C for 20 min, and washed. The dissociated implants were then resuspended in staining buffer (3% FBS, PBS), filtrated through a cell strainer 70µm nylon mesh membrane (BD discovery labware). The expression of the GFP reporter allows for the FACS analysis of progeny cells derived from input GFP-expressing MSCs from a GFP-negative host-derived cellular response.

5. Immunohistochemistry and quantification of Blood Vessel Density

At the time of implant harvest (15 days post-implantation), one mouse from each group had 1% paraformaldehyde injected into the left ventricle prior to sacrifice. The implants were removed and placed in 1% paraformaldehyde for 24 hours. Subsequently, they were fixed in 10% buffered formalin, and embedded in paraffin to be sectioned (5um). The sections were stained with isolectin B4 using the Vectastatin® Elite ABC system (Vector labs). Vascular density was assessed histologically with the aid of an Olympus BX60 microscope. The number of vessels (defined as tubular structures within the Matrigel with a patent lumen and lined with endothelium) was tallied and the vascular density was expressed as blood vessels/high power field (BVHPF). The mean number of

blood vessels per 5 (×100 magnification) fields of view was quantified by an independent observer in a blinded procedure.

6. Microarray Analysis

Total cellular RNA was extracted was from cultured MSCs by placing cell pellets in lysis buffer, homogenized and applied to RNA purification columns according to manufactures instructions (RNeasy®, Qiagen, Missassauga, ON, Canada). After washing the columns, the bound RNA was treated with DNAse I, washed and eluted. Reverse transcription, labeling and array hybridizations were performed by the microarray facility at the Ontario Genomics Innovation Centre (OGIC) within the Ottawa Health Research Institute (OHRI). Biotin-labeled complementary RNA was purified, fragmented, and hybridized to Affymetrix Murine Genome U74v2 chips (Affymetrix, Santa Clara, California), which contains ~30,000 mouse genes (n = 3 for each group).

Scanned raw data were processed with Affymetrix GeneChip version 5.0 software. The average intensity value for each probe set, which directly correlates to messenger RNA abundance, was calculated as an average of fluorescence differences for each perfectly matched probe versus single-nucleotide-mismatched probe. This software also gives each gene a qualitative assessment of "absent" or "present" calls. Data sets on each GeneChip were then imported into BRB ArrayTools version 3.2.2, developed by Dr. Richard Simon and Amy Peng Lam, (http://linus.nci.nih.gov/BRB-ArrayTools.html) for further analysis. When being imported, an intensity filter removed all genes with

a intensity value below 500, followed by a detection call filter that removed probesets with detection calls that have a value of "absent" in 50% or more of the samples. After these filters were applied the data was log transformed and subjected to median normalization. The gene expression database was then subjected to a variety of analysis tools (i.e. scatterplot, clustering gene ontology comparison), a differential gene expression of 1.5-fold or more between CD34+ and CD34- MSC was considered significant.

7. Statistics

All results are expressed as means ± standard error of the mean (s.e.m). Proportions were compared using Chi-square test and student's t-test was used for continuous variables. All statistical analysis was performed using SPSS data analysis software.

Results

Phenotype of cultured Marrow Stromal Cells and clonal subsets

MSCs derived from C57Bl/6 mice were retrovirally labeled to express GFP, with over 97% of these cells demonstrating this marker as demonstrated by flow cytometry. These GFP+ MSCs were subsequently analyzed for expression of commonly reported markers of endothelial progenitors and stem cells. As demonstrated by figure 1a, the phenotype of the polyclonal MSC population was: 15% CD34+, >96% CD44+, and did not express: CD13, CD31, CD45, CD117, Flk1, Flt3 and Tie2. We isolated twenty homogeneous clonal MSC subsets and analyzed expression of the CD34 antigen. We were able to detect CD34 expression in 3 of 20 clones analyzed and the level of CD34 expression amongst these clones was variable (figure 1b). The expression of all other markers was the same as in the parental mixed population from which they were derived (data not shown). The MSC clonal population with the highest CD34 expression level (hereafter MSC/CD34^{+}) and a randomly chosen MSC/CD34^{null} clonal subset were used for all subsequent experiments. Note that both of these clones were negative for the vascular markers, analogous to the parent heterogeneous population.

CD34 expression and MSC vascular plasticity *in vivo*

We have previously reported that Matrigel-embedded MSCs elicit a robust angiogenic response in normal mice.(4) We here use this same assay to determine whether CD34 expression by MSC clonal subsets correlates with

vascular plasticity and host-derived angiogenic response. In the following series of experiments, two GFP-labeled MSC clonal subsets – MSC/CD34$^+$ and MSC/CD34^{null} - were compared to mouse embryonal fibroblasts for their *in vivo* effects. As detailed in materials and methods, four million MSCs were admixed in 0,5 ml of Matrigel and the plug injected subcutaneously in normal C57Bl/6 mice (n=4 per group). At 15 days post-implantation, mice were sacrificed and matrigel plugs examined *in situ*. In Figure 2, representative examples of matrigel plugs in mice are shown. We observed that macroscopic neovascularization of the plugs occurred only with MSC implants, whereas matrigel only or fibroblast-containing matrigel plugs were consistently avascular.

Matrigel implants were surgically resected and collagenase-dissociated to allow for quantification and analysis of cellular content. The total number of nucleated cells isolated from collagenase dissociated Matrigel implants varied amongst the groups (figure 3a). A significantly greater number of cells were retrieved from the MSC/CD34$^+$, MSC/CD34^{null}, and embryonic fibroblast implants when compared to the Matrigel-alone implant (4.2±0.7 vs 1.9±0.5 vs 2.4±0.2 vs 0.2±0.1 x 10^6 cells respectively; $p<0.05$). However, the largest amount of cells was recovered from the MSC/CD34$^+$ implant when compared to all other groups ($p<0.05$). Cells retrieved from the dissociated Matrigel plugs were subjected to flow cytometric analysis. The expression of the GFP reporter allowed us to identify input MSCs and their differentiated progeny, whereas all GFP^{null} events were deemed to be host-derived. Recall that over 97% of the input cells were GFP expressing. As demonstrated by figure 3b, the percentage of GFP-

expressing cells of total was 1,1% for MSC/CD34$^+$ and 2,5% for MSC/CD34^{null} implants, respectively. The great majority of cells populating the Matrigel are host-derived (>97%). Analysis of the GFP-expressing cells for CD31 (PECAM) marker expression was performed to assess vasculogenic plasticity of MSCs (Figure 3c). We found that a subset of both MSC/CD34$^+$ and MSC/CD34^{null} cells expressed CD31 *de novo*. As shown in Figure 3d, MSC/CD34$^+$ generated significantly more CD31-coexpressing progeny than MSC/CD34^{null} (10.7±8.4% vs 3.1±0.6% respectively; $p<0.05$). As can be extrapolated from the above data, we found that less than 2% of input GFP-expressing MSCs remained detectable by day 15, a feature shared by both MSC/CD34$^+$ and MSC/CD34^{null} cells. To test whether cell death was involved in the observed loss of MSCs, we enumerated the number of live MSCs maintained in a Matrigel plug kept in a humidified incubator. Analysis of dissociated Matrigel revealed that up to 90% of MSCs are lost within 72 hours (data not shown).

Hematopoietic and endothelial host-derived cellular response to MSCs

When the total cellular content from the dissociated Matrigel implants were subjected to flow cytometric analysis, FSC/SSC scattergrams revealed that multiple distinct cellular subsets populate the implant. Amongst these GFP^{negative} cells the vast majority were CD45$^+$ hematopoietic cells, followed by CD45$^-$/CD31$^-$ and lastly CD45$^-$/CD31$^+$ cells that were likely endothelial in origin (Figure 4a). Host-derived endothelial cells were here defined as GFP^{negative}/CD31$^+$/CD45$^-$ and we used this as a surrogate measure of host-derived angiogenic response to

implanted MSCs. Figure 4b reveals that the mean proportion of host-derived endothelial cells recruited by either MSC/CD34^{+} or MSC/CD34^{null} implants was found to be significantly ($p<0.05$) greater than that associated with embryonal fibroblasts or Matrigel only. MSC/CD34^{+} implants led to the greatest host endothelial response when compared with MSC/CD34^{null} (4.2±0.5% vs 2.8±0.6%; $p<0.05$).

MSC subsets and Matrigel plug vascularity

Histochemical staining with isolectin B4 of fixed and sectioned Matrigel plugs was performed to identify capillaries within the implants at 15 days (figure 5a). The mean capillary density for each test group was assessed. Figure 5b shows that capillary density in MSC/CD34^{+} or MSC/CD34^{null} implants was found to be significantly ($p<0.05$) greater than that associated with embryonal fibroblasts or Matrigel. MSC/CD34^{+} implants led to the highest capillary density when compared with MSC/CD34^{null} (132 ± 22 vs. 85 ± 17 BVHPF $p<0.05$).

MSC CD34 Expression and Differential Gene Expression.

Total RNA was extracted from three distinct sets of culture expanded MSCs were processed as described in Methods and analyzed with Affymetrix Murine Genome U74v2 chips (Affymetrix, Santa Clara, California), which contains ~30,000 mouse genes (n = 3 for each group). A total of 1244 genes showed at least a 1.5 fold difference between the CD34+ and CD34- MSCs, 729 genes were higher in CD34+ cells, while 515 genes were higher in CD34- cells

(Figure 6). For a complete list of differential gene expression between CD34+ and CD34- MSC see data supplement 1. Established pro-angiogenic genes such as the VEGFs, angiopoietins, and FGF2 were not significantly altered between CD34+ and CD34- cells (Table 1), however several well established angiogenesis-related genes including FGF7, and hepatoma-derived growth factor, were significantly elevated in CD34+ cells (Table 1). In addition to these well-established angiogenesis-related genes, several angiogenesis-associated genes were also increased in the CD34+ cells (Table 1).

Discussion

In a previous study(4), we have demonstrated that MSCs from C57Bl/6 mice can undergo vasculogenesis *in vitro*, and induce a mostly host-derived, VEGF-dependent, neovascularization *in vivo.* MSCs differ from other reported vasculogenic EPCs in several aspects. Unlike EPCs, we and others(5) have shown that endothelial markers such as CD31/PECAM-1 are absent in MSCs and that in distinction to related MAPCs(10;11), CD34 is expressed by a subset of cultured C57Bl/6 MSCs. Also, there is no expression of the VEGF receptor Flk1, such as observed in MAPCs and EPCs. Therefore, MSCs represent a distinct stem cell subset to EPCs, yet share vasculogenic properties which may be exploited clinically. The expression of CD34 on culture expanded MSCs is a well described property in inbred mouse strains(5), in particular C57Bl/6 – a frequently used animal model of regenerative medicine. CD34 expression is widely utilized as a surrogate marker of stem cells in an array of clinical and pre-clinical regenerative medicine applications. In the field of cardiovascular regeneration in particular, the use of CD34 marker expression often defines and limits the cellular materials - either marrow or blood-derived EPCs - under study(6-9). The underlying assumption being that CD34 defines a wide subset of cells within which cardiovascular plasticity potential exists. However, marrow-derived, anchorage-dependent, $CD34^{null}$ mesenchymal adult progenitor cells (MAPCs) can also acquire endothelial markers *in vitro* with growth factor stimulation and will participate in cancer-associated angiogenesis *in vivo.*(10;11) Therefore, the utility of CD34 as a surrogate marker of vasculogenic plasticity

may vary with the type of blood/marrow stem cell under study, and this needs to be defined in MSCs.

To answer this question, we isolated homogeneous clonal populations of $CD34^+$ and $CD34^{null}$ MSCs and analyzed their post-natal vasculogenic and neo-angiogenic potential. We characterized 20 clonal MSC sets and found 3 which expressed CD34, all the others being $CD34^{null}$. The average level of expression of CD34 varied widely between the three $CD34^+$ clonal populations, but was homogeneous within each clonal population. All MSC clones studied did not express the CD45 hematopoietic marker or CD31/PECAM-1, a mature endothelial marker. We chose to further characterize *in vivo* the MSC subset which expressed the highest level of CD34 and compared its vasculogenic and angiogenic behavior to a randomly chosen $CD34^{null}$ MSC clonal subset. Our results clearly reveal that MSCs will undergo vasculogenic differentiation and acquire CD31/PECAM-1 *in vivo* and initiate a robust host-derived angiogenic response independently of their CD34 status prior to implantation. However, MSC/$CD34^+$ were significantly more efficient than MSC/$CD34^{null}$ at this process. We noted that the majority of input MSCs is lost from the implant after 14 days. *In vitro* experiments suggest that most of this loss may be due to demise of the MSCs following their harvest and admixture in Matrigel within the first 72 hours due to apoptosis. We may speculate that cell survival signaling may be disrupted when anchorage-dependent MSCs are suspended in matrix lacking appropriate microenvironmental cues.

We were also able to characterize host-derived cells that were recruited by MSCs to the implants. The vast majority of these cells were $CD45^+$ hematopoietic cells (approximately 65% of total) and a small percentage of infiltrating cells were host-derived endothelial cells. We show that MSC clonal populations exhibited a significant advantage in recruiting host endothelial cells over controls such as fibroblasts. It remains possible that the origin of these host endothelial cells may actually be from circulating EPCs that underwent post-natal vasculogenesis *in situ*. It was interesting to find that embryonic fibroblasts also recruited host endothelial cells though significantly less than that recruited by MSCs and may represent a non-specific host response to syngenic cellular implants. Previous studies have demonstrated that fibroblasts are known to elaborate growth factors under hypoxic conditions which are likely to exist in the Matrigel implant assay, and these mechanisms may be at work in our system.(12;13)

Our data strengthens our prior observation that MSCs will differentiate in to endothelial cells *in vivo,* and we now demonstrate that this potential exists irrespective of their CD34 status, at least in C57Bl/6 mice. We have focused on CD34 expression as a putative marker for vascular plasticity since it is the only hemagiopoietic marker -amongst those we tested as part of this report - whose expression levels differs between murine MSC clonal subsets. It is conceivable that in addition to CD34 other MSC cell surface marker such as CD117(14) or CD140a(15) may predict for enhanced vascular plasticity *in vivo*. However, the MSCs we studied did not express CD117 and the significance of this marker in

our and other vasculogenic systems remains to be defined(16). We have also observed that the bulk of the *in vivo* angiogenic response to MSCs, independently of their CD34 status, is host-derived. This strongly supports the notion that MSCs mediate their *in vivo* angiogenic effect via a paracrine mechanism(4;17). In contrast to the use of enriched "$CD34^+$ peripheral cells" as has been championed in pre-clinical and clinical cardiovascular cellular regenerative medicine studies(8;18), lack of CD34 expression does not adversely affect the vasculogenic capabilities of MSCs in mice. This observation highlights the inadequacy of CD34 expression as a universal surrogate marker for stem cells capable of vascular regeneration and caution must be exercised in cell regeneration research by omitting important yet unrecognized $CD34^{null}$ stem cell subsets as here described.

Despite the fact that CD34 expression does not preclude vasculogenic potential of MSCs, CD34+ MSCs did created a denser and thus more complex vascular network. This may be due to functional differences in the two clonal populations particularly in their ability to stimulate the host-derived angiogenic response. To gain insight into potential mechanisms underpinning these differences, we compared the gene mRNA profiles of CD34+ and $CD34^{null}$ cells *in vitro*. Our analyses clearly distinguished the two populations as evidenced by the 6 fold difference in CD34 expression we found between the cell types (See supplemental data set). In total we found 1244 genes to be differential regulated between the groups, but focused our attention on those genes which were increased in the CD34+ cells and were secreted proteins. From this list,

candidate genes were further selected according to literature evidence of their ability to act as angiogenic factors. From this we identified four growth factors (FGF7, HDGF, NGFb, and BDNF) which can influence angiogenesis.(19-27) In particular, FGF7 and HDGF are interesting targets as protein expression for both these mediators have been identified in the media of marrow-stem cells.(17;28) We also identified an increase in expression of the extracellular matrix protein Tenascin C, which influences angiogenesis through VEGF(29;30) and Glypican can also influence the actions of VEGF(31), while Apelin is a recently established VEGF-independent angiogenic factor.(32) Together these results suggest that modulation of the extracellular environment by CD34+ may enhances the host derived vascular response. It is of particular interest that there was no significant difference in the expression of VEGF A and B irrespective of their CD34 status. The shared VEGF gene expression profile may explain the ability of $CD34^{null}$ MSCs to initiate angiogenesis and conciliates with our prior observation that VEGF is an essential component in this phenomena *in vivo.*(4)

Indeed, there are other reports describing the contribution of CD34-negative peripheral blood stem cells to vascular regeneration.(33;34) Based on our results, we surmise that CD34 in itself play little to no direct role in neovascularization, especially since the CD34 knock-out mice had no defined vasculopathic phenotype.(35) In humans, the expression of CD34 on human fetal stromal progenitors has been reported, though the frequency of CD34-expressing stromal progenitors decreases as gestational age progresses(36). Postnatal human marrow stromal precursors can also be recovered within the

CD34 fraction of marrow nucleated cells(37). However, there is broad consensus that culture-expanded human MSCs do not express the CD34 antigen(38;39). We must conclude that $CD34^{null}$ marrow-derived stem cells, including MSCs, will likely lead to a clinically robust, host-derived, angiogenic response in those anatomical compartments - such as heart and limb - where therapeutic neovascularization would be clinically desirable.

Appendix 1

Table 1

CD34+ Marrow Stromal Cells Proangiogenic Gene Expression

	Log Gene Expression CD34+	CD34-	Fold Induction	P value	Ref.
Pro-angiogensis					
Angiopoeitin-1	< MIN	< MIN	N.D.	N.D.	
Angiopoeitin-2	< MIN	< MIN	N.D.	N.D.	
VEGFA	10.309	10.088	1.022	0.546026	
VEGFB	11.671	11.578	1.008	0.633991	
VEGFC	<MIN	<MIN	N.D.	N.D.	
FGF1	<MIN	<MIN	N.D.	N.D.	
FGF2	<MIN	<MIN	N.D.	N.D.	
Angiogenesis-related					
FGF7	11.321	10.090	2.347	0.000957	
Hepatoma-derived growth factor	14.549	13.905	1.563	0.003638	
Angiogensis-assoicated					
Nerve growth factor, beta	10.655	9.462	2.286	0.00265	
Brain derived neurotrophic factor	12.641	11.904	1.666	0.000646	
Tenascin C	9.901	9.127	1.711	0.004015	
Glypican 1	13.043	12.220	1.770	0.00888	
Apelin	13.113	12.182	1.906	0.002499	

Figures

Phenotypic Characterization of MSCs

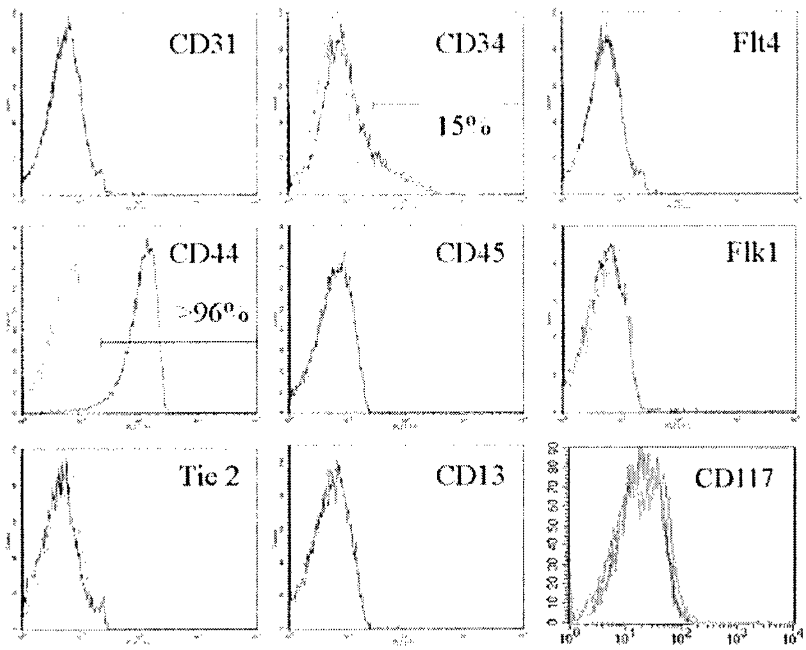


Figure 1a

Variable Expression of CD34 Antigen in Clonal Populations

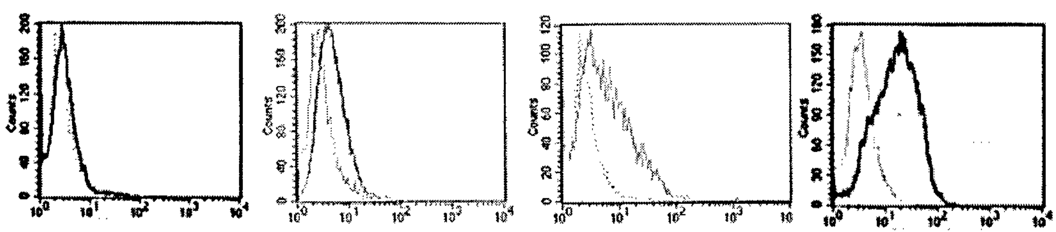


Figure 1b

Appendix 1

Figure 1

MSC phenotypic analysis. Figure 1(a) reveals the flow cytometric analysis our mixed population of MSCs from which clonal subsets was isolated. Expression profiles for CD13, CD31, CD34, CD44, CD45, flk1, flt4 and Tie2 were performed as detailed in materials and methods. The fraction of positive events is indicated in those analyses that had detectable marker expression. Figure 1(b) demonstrates the variable expression of the CD34 antigen amongst MSC clones isolated via limiting dilution. The left panel depicts the analysis of a representative CD34-negative clone, the three panels to its right reveal the level of CD34 expression in the three clones where its expression was detectable. *In vivo* studies were performed with the clone with highest average expression level of CD34 (last panel on right). Specific antibody profile (solid line) versus isotype-matched antibody control (hatched line) is depicted.

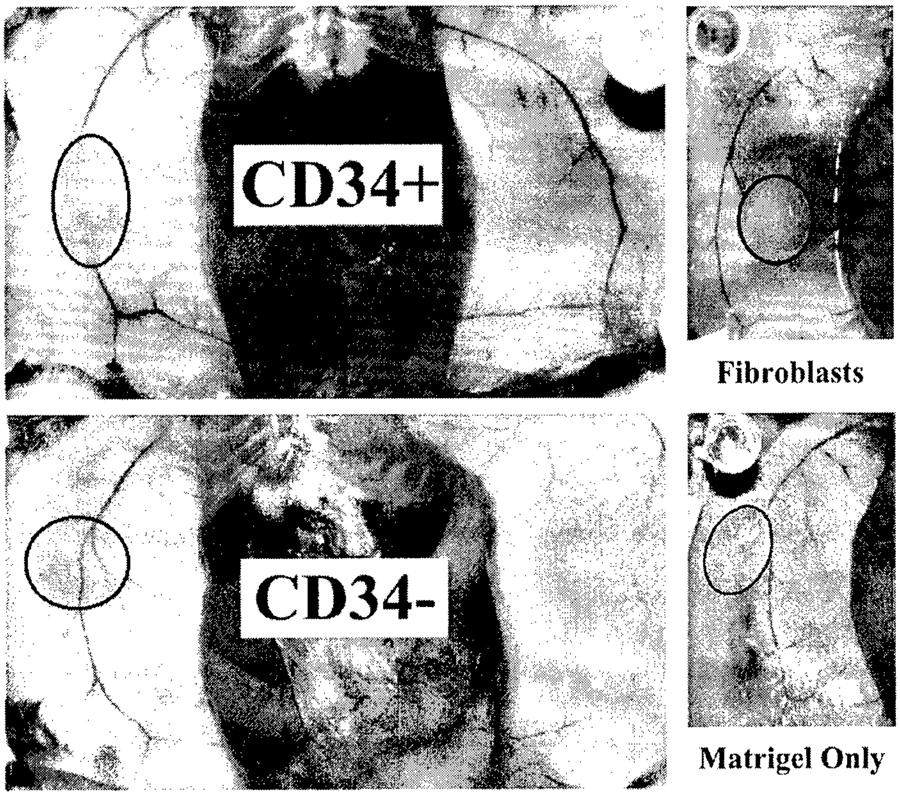


Figure 2

Figure 2

Matrigel plug assay *in vivo* – gross macroscopic appearance. At 15 days post matrigel plug implantation, experimental mice were sacrificed and subcutaneous plugs exposed. Representative examples are shown of mice implanted with MSC/CD34^{+} (top left panel) and MSC/CD34^{null} (lower left panel). There was clear gross evidence that neovascularization had taken place within and about the MSC matrigel implants. In contrast, the fibroblast (top right panel) and matrigel-only (lower right panel) implants appeared relatively avascular.

Appendix 1

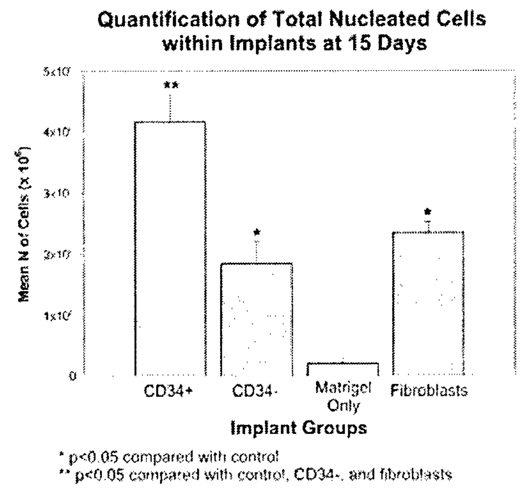


Figure 3a

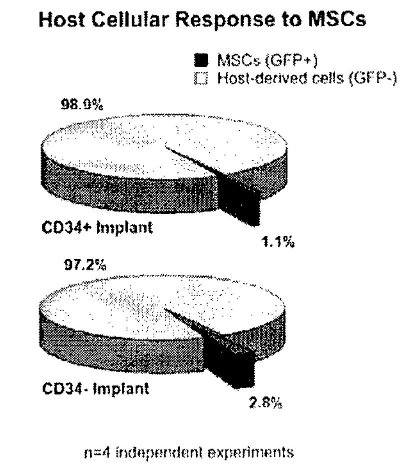


Figure 3b

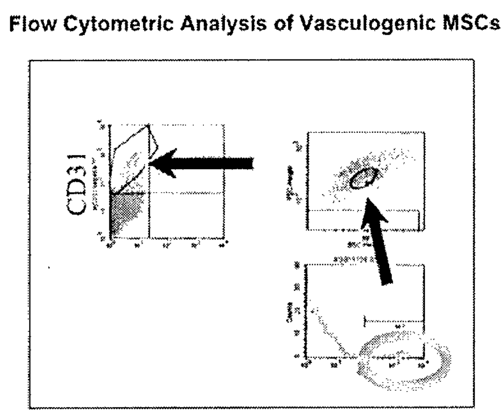


Figure 3c

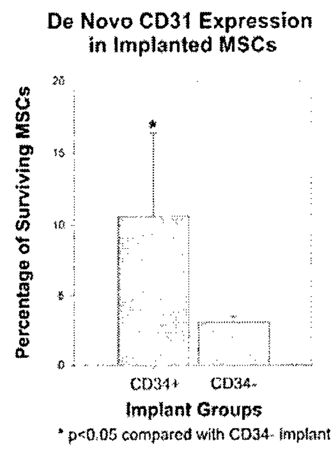


Figure 3d

Figure 3

Analysis of GFP-labeled MSCs and their progeny in dissociated matrigel pugs. Matrigel plugs from individual mice were harvested at 15 days post-implantation and dissociated into single-cell preparations. The total mean number of cells retrieved from each implant group (n=5 per group) is represented in fig 3(a). Fig 3 (b) depicts the proportion of GFP positive cells relative to all live cells retrieved from dissociated MSC matrigel plugs. Total cells retrieved from each MSC-containing matrigel plug was labeled for CD31 and analyzed for co-expression of GFP and a representative example of analysis is shown in Fig 3 (c), whilst the aggregate data for each test group (n=5, average± sem) is depicted in Fig 3 (d).

Characterization of Cellular Infiltrate within Matrigel Implants

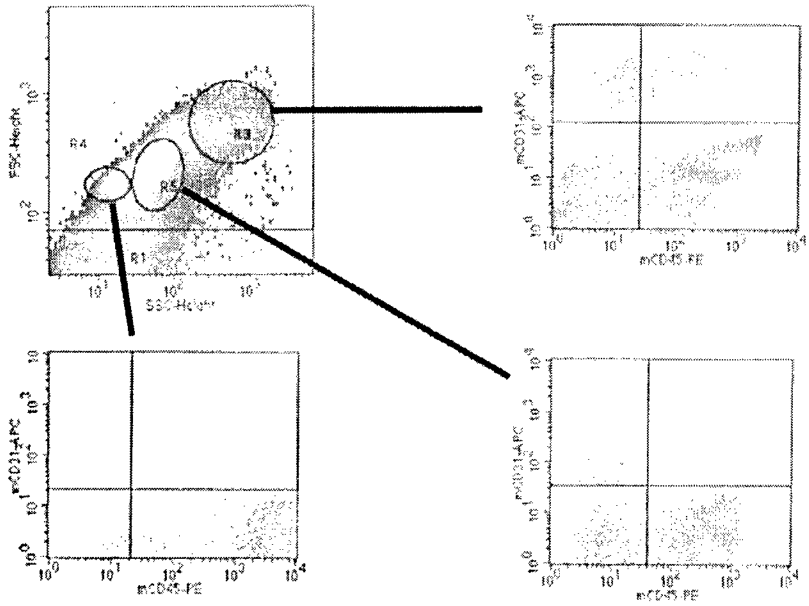
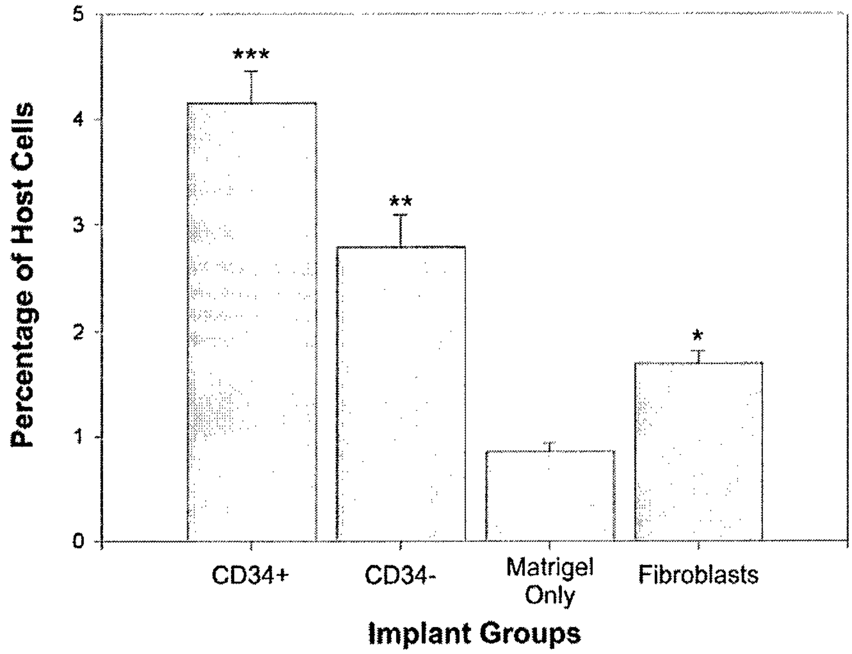


Figure 4a

Host-derived Endothelial Cells



* p<0.05 compared with control
** p<0.05 compared with control, and fibroblasts
*** p<0.05 compared with control, fibroblasts, and CD34-

Figure 4b

Figure 4

Analysis of CD31-expressing cells in dissociated matrigel plugs. Using CD45 and CD31 co-labeling and gating on dominant populations defined by FSC/SSC profile, we queried the content in hematopoietic cells ($CD45^{+}/CD31^{-}$ & $CD45^{+}/CD31^{+}$) and endothelial cells ($CD45^{-}/CD31^{+}$). A representative analysis is depicted in fig 4(a) and reveals the multiple host-derived cell types are present within the implants at 15 days post-implantation. The proportion of $CD45^{-}/CD31^{+}$ endothelial cells for each test group (n=5, average± sem) is represented in fig 4(b).

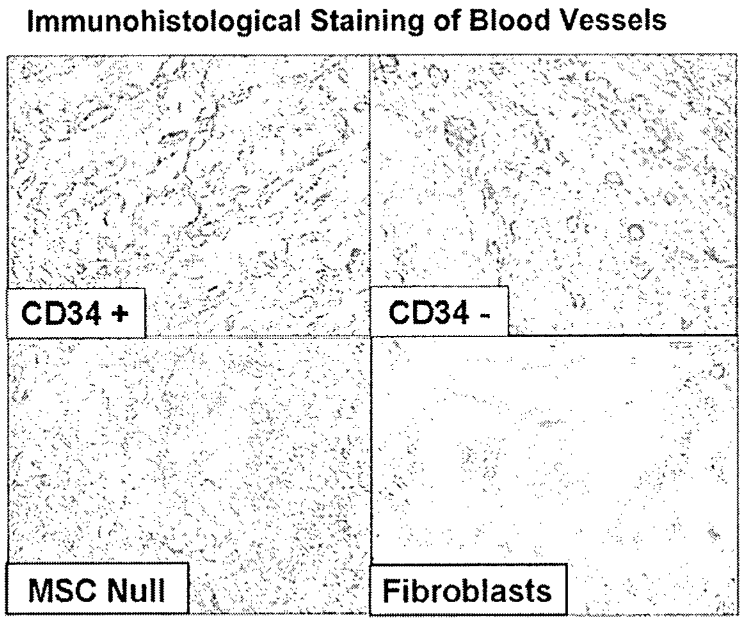
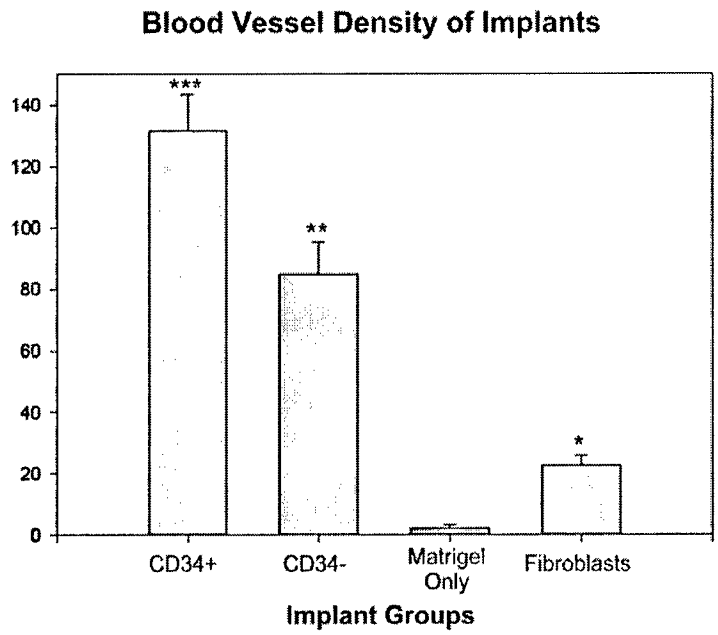


Figure 5a



* $p<0.05$ compared with control
** $p<0.05$ compared with control, and fibroblasts
*** $p<0.05$ compared with control, fibroblasts, and CD34-

Figure 5b

Figure 5

Histologic analysis of angiogenesis in excised matrigel plugs. Vascular density within each implant group was performed using isolectin B4 immunostaining as described in materials and methods. Blood vessels were defined as tubular structures within the matrigel with a patent lumen and lined with endothelium. The vascular density was expressed as blood vessels/high power field (BVHPF). Representative histological sections (magnification x100) are shown for each test group (panel 5a) and average blood vessel density for each test group (n=5, average± sem) is represented (panel 5b).

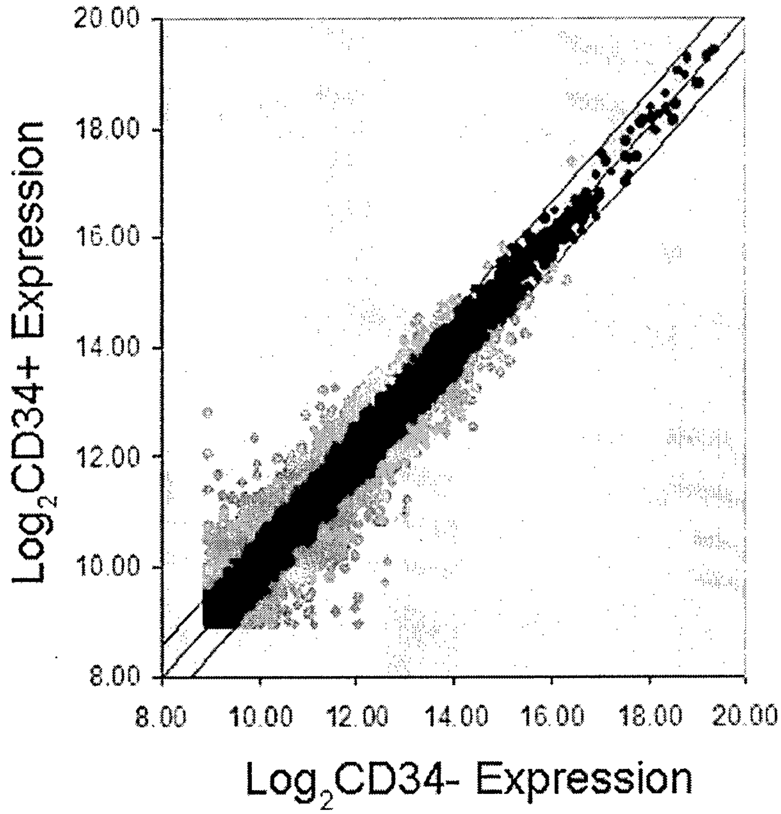

20.00
18.00
16.00
14.00
12.00
10.00
8.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
Log$_2$CD34+ Expression
Log$_2$CD34- Expression

Figure 6

Figure 6

Scatterplot of CD34 phenotype class based on differential gene expression. By , ploting the average log-ratio of each gene that passed our filtering criteria for CD34- MSCs on the x-axis versus the average log-ratio of each gene that passed our filtering criteria for CD34+ on the y-axis we compared compare the differential gene expression patterns in CD34+ vs. CD34- MSCs. The majority of genes were plotted as points that line up along a 45-degree diagonal line (black points), while genes that are differentially expressed between the two CD34+ and CD34- phenotypes fall outside the outlier lines (red points). These outlier lines indicate genes for that the fold-difference between the geometric mean of the expression ratios within each of the two classes is greater 1.5.

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Appendix 1

CD34+ vs CD34- Marrow Stromal Cells Differential Gene Expression
DATA Supplement

Gene Symbol Probe Set	Log Gene Expression CD34+	 CD34-	Fold Induction
Increased in CD34+			
H19	12.792	8.966	14.182
Gatm	12.035	8.966	8.396
Brunol4	11.662	9.158	5.674
Ankrd1	12.348	9.922	5.373
Gatm	11.385	8.966	5.349
D3Ertd330e	11.269	9.274	3.984
Klra18	11.862	9.966	3.720
Il1rl1	11.517	9.666	3.607
Aqp1	13.180	11.332	3.599
Mlp	10.768	8.966	3.487
4930452B06Rik	12.765	10.975	3.459
Sfrp2	11.237	9.479	3.383
Idb2	10.717	8.966	3.365
Il1rl1	11.057	9.328	3.317
Cd34	10.692	8.966	3.309
Cd34	10.676	8.966	3.271
Ddah1	11.651	9.960	3.230
D3Ertd330e	10.914	9.266	3.135
Krt1-19	12.920	11.301	3.071
117151_at	13.216	11.618	3.028
129778_at	10.903	9.334	2.967
B130017I01Rik	11.939	10.370	2.966
Chn1	10.506	8.966	2.909
108505_at	10.699	9.167	2.893
Scara3	11.953	10.439	2.856
Eps8	11.133	9.621	2.853
Tpd52l1	11.246	9.742	2.836
Klra23	10.458	8.966	2.814
2610318C08Rik	10.533	9.104	2.693
Mfap3l	10.659	9.236	2.682
Plk4	12.056	10.700	2.559
Gzmb	10.784	9.438	2.544
Gp38	11.260	9.932	2.511
Osp94	12.808	11.482	2.506
Slk	10.431	9.111	2.497
Pkia	11.040	9.721	2.496
Mfap3l	12.836	11.525	2.482

Myh10	10.359	9.053	2.473
2610027C15Rik	12.453	11.151	2.465
Ect2	12.192	10.893	2.460
Klf5	10.446	9.148	2.459
Abcg2	10.521	9.232	2.443
2810406C15Rik	12.150	10.862	2.442
Gnb4	11.326	10.052	2.420
Grem2	10.276	9.009	2.408
163825_at	12.681	11.416	2.403
116194_at	10.671	9.406	2.402
Bub1	10.682	9.420	2.399
Gm1805	10.504	9.247	2.390
Osp94	11.894	10.637	2.389
Mmp23	10.946	9.692	2.385
2310066N05Rik	10.481	9.228	2.383
Fgf7	11.321	10.090	2.347
3830403N18Rik /// Xlr	10.619	9.395	2.336
103203_f_at	10.323	9.101	2.332
Cugbp2	10.847	9.626	2.331
Myod1	10.996	9.790	2.307
Gnb4	11.078	9.877	2.300
164090_i_at	10.909	9.709	2.299
Osbpl3	12.091	10.891	2.297
105694_at	11.043	9.845	2.295
Ngfb	10.655	9.462	2.286
99959_at	10.546	9.357	2.281
Rg9mtd2	10.585	9.396	2.280
2610036L11Rik	12.081	10.893	2.278
Fdps	14.427	13.241	2.275
Inhba	12.012	10.835	2.261
4933434E20Rik	14.513	13.338	2.258
1810033A06Rik	11.776	10.609	2.246
Trim33	12.609	11.447	2.238
3732409C05Rik	10.799	9.637	2.237
1700108L22Rik	11.596	10.435	2.235
BC016226	10.886	9.732	2.225
0610010O12Rik	11.531	10.385	2.214
Fdps	13.559	12.414	2.211
Btbd3	11.387	10.244	2.210
Rtcd1	13.168	12.029	2.203
Spag5	11.231	10.094	2.199
AI663987	12.391	11.262	2.187
Snx7	13.482	12.356	2.183

BB128963	10.335	9.213	2.176
BC020354	12.988	11.867	2.175
Pkn2	11.393	10.278	2.166
B230339E18Rik	10.804	9.691	2.163
4930579G24Rik	12.563	11.453	2.159
Slco2a1	12.955	11.848	2.154
108749_at	10.504	9.403	2.145
Rrm2	11.489	10.393	2.138
Sdpr	13.308	12.213	2.136
5133401H06Rik	10.533	9.440	2.132
Rtn2	10.051	8.966	2.121
111203_at	10.689	9.606	2.118
2700084L22Rik	10.092	9.010	2.117
Idi1	12.251	11.169	2.117
137494_f_at	11.029	9.947	2.117
117265_at	10.783	9.703	2.115
Mthfd1	11.597	10.519	2.112
Mllt3	11.379	10.301	2.111
Ddah1	11.017	9.955	2.089
Narg1	10.305	9.244	2.086
Sod3	11.268	10.211	2.081
Gemin6	11.878	10.822	2.079
Cks1	14.073	13.017	2.079
B230209C24Rik	12.335	11.282	2.074
Pcdh18	11.953	10.901	2.073
2810052M02Rik	10.341	9.294	2.066
1300014I06Rik	12.634	11.589	2.064
Il13ra1	10.609	9.565	2.062
96016_at	12.496	11.453	2.061
5430428G01Rik	11.778	10.745	2.046
Mad2l1	12.414	11.389	2.035
Anln	13.097	12.075	2.031
Mybbp1a	11.022	10.001	2.030
Itga3	11.492	10.471	2.030
Hells	10.417	9.397	2.028
Lss	10.735	9.715	2.027
138147_at	10.895	9.876	2.026
Taf10	12.402	11.384	2.025
C330008K14Rik	11.567	10.551	2.023
Pmvk	10.945	9.929	2.023
170306_at	10.440	9.424	2.023
2810407C02Rik	12.808	11.793	2.021
Snrpd1	10.611	9.598	2.018
Nes	12.407	11.401	2.008

Acp1	10.087	9.081	2.008
Grwd1	10.358	9.352	2.008
Fshprh1	11.683	10.684	1.999
169431_r_at	10.633	9.636	1.996
Lrba	11.034	10.037	1.995
170664_i_at	10.424	9.429	1.993
Amd1	10.172	9.180	1.988
Bcap29	10.141	9.154	1.982
2610203C20Rik	10.960	9.973	1.981
Gmps	13.285	12.300	1.979
Tfrc	11.010	10.026	1.977
Sdpr	10.776	9.793	1.977
Gclm	11.996	11.016	1.972
112204_at	10.036	9.056	1.972
Pop5	11.549	10.572	1.968
Ube2q	11.583	10.609	1.964
Psma5	13.369	12.396	1.962
Igsf4a	10.003	9.031	1.962
Mpp6	12.412	11.440	1.961
1110020L19Rik	12.959	11.988	1.961
Brunol4	9.936	8.966	1.959
Dtymk	11.040	10.079	1.948
Dhx36	12.371	11.410	1.947
Baz1a	10.355	9.394	1.947
Anxa5	13.475	12.514	1.946
E030024M05Rik	11.201	10.240	1.946
Nrn1	14.745	13.786	1.945
AI326477	9.919	8.966	1.937
2400002F11Rik	10.624	9.671	1.936
D11Ertd172e	11.334	10.382	1.935
Usp15	11.188	10.238	1.931
Cd24a	11.158	10.209	1.930
BC026744	9.955	9.008	1.928
Spire1	9.918	8.972	1.927
108366_at	10.195	9.252	1.923
Ak4	10.169	9.226	1.922
Ccnc	11.716	10.775	1.919
4933434L15Rik	10.569	9.630	1.917
Pfdn4	11.814	10.876	1.916
Atp1a1	12.446	11.509	1.914
D17H6S56E-5	11.755	10.821	1.911
Idb1	10.754	9.821	1.909
2210016F16Rik	11.164	10.231	1.909
Kif20a	11.497	10.565	1.909

108957_at	10.131	9.198	1.908
4930467B06Rik	11.125	10.193	1.908
Apln	13.113	12.182	1.906
92564_at	10.802	9.871	1.906
Ifrd1	11.923	10.996	1.901
Abcg2	10.349	9.427	1.894
Bpgm	10.517	9.596	1.893
Maff	12.623	11.703	1.893
5830433M19Rik	10.216	9.295	1.893
Pmf1	11.296	10.377	1.891
Anxa3	13.863	12.945	1.889
165862_f_at	17.397	16.480	1.888
Tfrc	11.204	10.288	1.887
Nsdhl	11.056	10.144	1.882
Stard4	12.068	11.157	1.881
5730507H05Rik	11.489	10.579	1.879
Ndufc1	12.294	11.384	1.879
Mrpl11	10.137	9.231	1.874
Osr1	12.193	11.287	1.873
Hccs	11.949	11.044	1.873
1700029F09Rik	11.405	10.500	1.873
2610318C08Rik	11.324	10.421	1.871
Stard4	12.944	12.041	1.870
Prpf3	10.849	9.946	1.870
Strbp	10.248	9.347	1.867
2310022M17Rik	12.476	11.576	1.866
Ptgs2	11.743	10.844	1.865
Plaur	10.531	9.633	1.864
2600014C01Rik	10.548	9.650	1.863
Baz1b	10.497	9.600	1.863
Ilf2	10.432	9.536	1.861
2700019D07Rik	10.723	9.827	1.861
Rabggtb	12.001	11.107	1.858
Sec24b	11.128	10.235	1.858
Anp32e	11.937	11.043	1.858
Kntc1	10.593	9.701	1.856
114299_at	14.206	13.315	1.855
Rangap1	11.544	10.653	1.854
Denr	14.592	13.701	1.854
2010204K13Rik	12.383	11.494	1.852
2010309E21Rik	10.435	9.548	1.850
Csnk2a1	9.996	9.111	1.847
129017_at	13.627	12.743	1.845
Cdk4	12.658	11.775	1.843

1200008O12Rik	11.592	10.711	1.842
4121402D02Rik	10.775	9.894	1.841
1110013G13Rik	10.028	9.148	1.841
Clspn	10.717	9.837	1.840
Stk6	11.748	10.868	1.840
168384_s_at	12.111	11.233	1.837
98602_at	12.355	11.478	1.837
0610025O11Rik	12.008	11.131	1.836
Pop4	10.857	9.984	1.831
Pdzk11	11.948	11.076	1.830
129223_at	12.211	11.339	1.830
1110007F05Rik	12.605	11.734	1.830
Commd10	12.283	11.412	1.829
164085_at	9.956	9.085	1.829
Acad9	10.510	9.642	1.825
139044_at	10.343	9.477	1.823
2700069A02Rik	12.014	11.149	1.821
Gja1	11.478	10.614	1.821
Phf17	10.544	9.680	1.820
Ddah1	11.260	10.397	1.820
Phf17	12.274	11.411	1.819
Purb	10.857	9.995	1.818
Prkcm	9.844	8.984	1.815
Gfm	10.727	9.869	1.813
2810433K01Rik	10.682	9.824	1.812
Ddx20	10.807	9.951	1.811
2610001J05Rik	9.994	9.139	1.809
D19Ertd678e	10.627	9.772	1.809
166966_at	11.946	11.091	1.809
113442_at	10.671	9.817	1.807
Ccnd1	12.019	11.167	1.805
Fpgt	10.989	10.138	1.803
Rras2	12.889	12.039	1.803
D6Ertd365e	10.674	9.824	1.803
Usp14	11.745	10.896	1.802
Snrpf	14.103	13.254	1.802
Mrpl17	11.144	10.295	1.801
110746_f_at	14.290	13.442	1.801
Kif2c	11.239	10.391	1.800
1500041J02Rik	11.090	10.242	1.800
4933434E20Rik	11.180	10.332	1.800
Cdc20	13.319	12.471	1.799
171518_i_at	13.644	12.798	1.798
Arch	10.753	9.909	1.796

Idi1	11.378	10.534	1.796
Gnb1	11.754	10.910	1.795
Acsl3	11.066	10.222	1.794
Pbk	13.446	12.605	1.792
Pdap1	13.745	12.904	1.791
1810055D05Rik	11.110	10.270	1.790
2310002J21Rik	11.689	10.849	1.790
Phca	13.240	12.400	1.790
Apln	11.250	10.410	1.789
1700034H14Rik	10.449	9.610	1.789
9030617O03Rik	11.697	10.861	1.785
S100a4	15.853	15.017	1.785
168975_i_at	14.740	13.905	1.783
Trim59	11.395	10.565	1.778
Prkar2b	11.634	10.806	1.774
Lmo7	10.438	9.611	1.774
2410018L13Rik	11.592	10.765	1.773
Acad9	12.603	11.779	1.771
Prss25	11.661	10.837	1.770
Gpc1	13.043	12.220	1.770
2310057G13Rik	10.036	9.212	1.770
2600017H08Rik	10.952	10.128	1.770
Chst1	12.172	11.349	1.770
Hbs1l	11.991	11.168	1.769
Sgcb	9.788	8.966	1.768
Foxg1	12.023	11.202	1.767
1700095N21Rik	12.831	12.010	1.767
Mrpl47	12.874	12.053	1.766
BC006933	9.787	8.966	1.766
2510048O06Rik	12.744	11.924	1.765
Egfl5	10.023	9.203	1.765
Lsm3	12.455	11.636	1.765
6720467C03Rik	13.856	13.039	1.762
9030408N13Rik	12.621	11.804	1.761
Polr3e	12.531	11.715	1.761
1110001A07Rik	10.972	10.156	1.760
Med6	13.925	13.109	1.760
2810468K05Rik	11.760	10.947	1.758
134778_at	11.531	10.718	1.757
Kif20a	12.975	12.163	1.755
95978_at	10.226	9.414	1.755
Wdr12	12.003	11.193	1.754
Pla2g12a	12.995	12.185	1.753
Mrps21	12.354	11.545	1.753

Tgfbi	9.774	8.966	1.752
1700029M07Rik	11.274	10.466	1.752
Lnpep	10.893	10.084	1.752
167619_r_at	11.505	10.697	1.751
S100a13	11.724	10.916	1.750
Whsc1	11.946	11.140	1.748
Gpd2	9.839	9.033	1.748
Dbi	13.385	12.580	1.748
Cugbp2	14.815	14.009	1.748
Plk1	12.488	11.685	1.745
Amd1	12.152	11.350	1.744
Egfl7	10.654	9.853	1.743
Rbm8	12.391	11.590	1.743
167824_f_at	12.388	11.588	1.741
Tnpo1	11.903	11.103	1.741
D5Ertd606e	10.986	10.187	1.740
Cdh2	11.318	10.520	1.738
4632434I11Rik	11.125	10.329	1.737
2310028O11Rik	13.419	12.623	1.737
Cdk2ap1	13.669	12.872	1.736
Rnf14	10.287	9.491	1.736
4933434E20Rik	11.753	10.959	1.734
Prkci	13.768	12.974	1.734
Lsm2	12.520	11.727	1.733
C330023M02Rik	10.549	9.757	1.731
Narg1	11.782	10.993	1.728
D7Ertd458e	10.174	9.386	1.727
Lrp12	10.278	9.491	1.725
4933434E20Rik	12.242	11.455	1.725
2610029K21Rik	9.828	9.043	1.723
4732479N06Rik	9.939	9.155	1.722
Strn3	13.285	12.501	1.722
112356_at	12.570	11.787	1.720
Ppp5c	9.918	9.135	1.720
Rrm1	12.085	11.303	1.720
171221_at	11.781	10.999	1.719
Slc35a3	10.687	9.906	1.719
Sin3b	12.465	11.684	1.718
Dusp19	10.286	9.506	1.718
Tfpt	10.688	9.908	1.717
1810043J12Rik	12.933	12.154	1.717
2810417H13Rik	14.377	13.597	1.717
AI314976	10.390	9.610	1.716
1700108L22Rik	11.764	10.986	1.715

Nsbp1	11.351	10.574	1.714
3110052N05Rik	10.831	10.054	1.714
Rdh10	11.607	10.830	1.714
Cugbp1	11.988	11.211	1.713
Rfxap	11.251	10.475	1.713
Odc1	11.312	10.536	1.711
Tnc	12.485	11.710	1.711
Prkcm	11.476	10.702	1.711
1810009A15Rik	11.602	10.828	1.710
Tnc	9.901	9.127	1.710
2810409H07Rik	11.305	10.531	1.710
Cited2	13.647	12.873	1.710
Shmt1	10.350	9.577	1.708
Cldn15	11.543	10.772	1.707
Ggh	9.747	8.976	1.707
Chc1	11.363	10.592	1.706
Alcam	10.271	9.501	1.706
Hoxa9	10.204	9.434	1.705
Epha2	10.998	10.228	1.705
Sema3a	12.502	11.733	1.704
6720463E02Rik	12.773	12.006	1.703
Cct3	14.059	13.291	1.703
Cdc2a	13.711	12.945	1.701
5730470L24Rik	11.599	10.835	1.699
Nsdhl	11.897	11.132	1.699
Snx27	12.174	11.410	1.699
Cmas	11.298	10.535	1.697
C130071C03Rik	11.638	10.874	1.697
Luc7l	12.053	11.290	1.697
Gmnn	10.695	9.932	1.697
Ppp1r12c	11.671	10.909	1.695
Magi1	10.439	9.677	1.695
2410012M04Rik	9.878	9.118	1.694
Uqcrh	13.103	12.343	1.694
Smc4l1	12.853	12.094	1.693
3110001D03Rik	13.124	12.366	1.691
Rasgrp3	9.867	9.109	1.691
S100a11	15.471	14.714	1.691
Kif4	11.116	10.358	1.690
Fignl1	11.685	10.930	1.688
Nola1	12.948	12.193	1.688
Zfp261	10.824	10.069	1.687
Ard1	11.370	10.616	1.687
Cul5	10.246	9.492	1.686

Magea2	10.103	9.350	1.686
Acate2	12.861	12.109	1.684
Khdrbs3	14.871	14.119	1.684
Mrpl54	10.783	10.033	1.683
Tnfaip1	10.351	9.600	1.683
Ivns1abp	10.644	9.893	1.682
Rnmt	11.926	11.177	1.681
Slc12a2	10.886	10.138	1.680
Tfrc	13.639	12.892	1.679
H2afz	14.194	13.446	1.679
Strap	12.090	11.342	1.679
Snrpa1	10.885	10.138	1.678
116938_at	12.918	12.173	1.677
Zzz3	10.962	10.216	1.677
1700007H16Rik	11.844	11.099	1.677
168697_s_at	12.047	11.302	1.676
1300002C08Rik	12.217	11.473	1.676
D19Ertd678e	11.293	10.550	1.674
Prkci	10.228	9.486	1.673
Eif1ay	13.802	13.060	1.673
133155_at	13.038	12.296	1.673
Commd2	11.645	10.903	1.673
Hnrpa2b1	10.689	9.947	1.673
4933421H10Rik	12.022	11.281	1.671
6230416J20Rik	10.380	9.639	1.671
Nsdhl	14.158	13.417	1.671
1110049G11Rik	10.933	10.193	1.669
Slc25a13	9.705	8.966	1.669
2210415M20Rik	10.109	9.371	1.668
Nbea	10.351	9.614	1.667
Bdnf	12.641	11.904	1.666
D5Ertd593e	10.361	9.626	1.665
Cugbp2	10.145	9.410	1.664
Mak3	12.599	11.865	1.663
Hat1	11.078	10.344	1.663
8030499H02Rik	10.438	9.704	1.663
9430083G14Rik	10.368	9.634	1.663
Amd1	11.895	11.161	1.662
Tnnt2	10.127	9.395	1.662
162799_at	9.698	8.966	1.661
Pole3	10.650	9.919	1.660
Tnpo3	12.199	11.468	1.660
Ube1x	14.195	13.465	1.659
Ahcy	13.115	12.387	1.657

Nup62	10.950	10.222	1.656
Kcnk2	9.693	8.966	1.655
Dbt	10.601	9.874	1.655
Tnfsf5ip1	10.319	9.593	1.654
2410075D05Rik	10.241	9.515	1.654
1110020G09Rik	9.824	9.099	1.653
BC065123	10.961	10.237	1.652
4930422J18Rik	10.601	9.878	1.650
Slc25a3	10.888	10.167	1.649
Rpa3	11.288	10.567	1.648
Prkrip1	10.353	9.632	1.648
101179_at	14.686	13.965	1.647
BC034664	11.743	11.024	1.646
166778_i_at	11.226	10.508	1.645
4632417K18Rik	12.069	11.352	1.644
Pop1	12.435	11.718	1.644
Lass2	10.933	10.216	1.643
Cetn2	9.872	9.155	1.643
Gkap1	10.198	9.482	1.642
Zfp265	13.555	12.839	1.642
Hmgb1	11.986	11.271	1.642
BC004701	11.638	10.923	1.641
2410066K11Rik	11.399	10.686	1.639
Sh3glb1	10.897	10.184	1.639
Atp11b	12.070	11.357	1.639
2410030K01Rik	11.185	10.472	1.639
Bcl2l1	11.902	11.189	1.638
Tpx2	12.776	12.065	1.637
Mtpn	13.080	12.369	1.637
Knsl7	11.453	10.744	1.635
105858_at	12.351	11.643	1.634
Pigm	9.914	9.207	1.633
Pitrm1	12.314	11.608	1.632
168120_r_at	10.522	9.817	1.629
Sytl2	10.622	9.918	1.629
Adrbk1	10.007	9.303	1.629
1810015C04Rik	11.966	11.263	1.627
Zwint	14.025	13.322	1.627
Acsl4	10.159	9.458	1.626
D7Ertd156e	12.883	12.182	1.625
130068_f_at	11.138	10.438	1.625
9130213B05Rik	10.873	10.174	1.623
167994_at	9.675	8.978	1.621
Rad51	11.649	10.954	1.620

Cdc25a	11.167	10.471	1.619
Strn3	11.290	10.596	1.618
Seh1l	11.719	11.025	1.618
Piga	9.765	9.071	1.617
Cct3	13.112	12.418	1.617
Sc4mol	12.222	11.529	1.617
Cklf	12.962	12.268	1.617
Taldo1	11.044	10.351	1.617
1300014I06Rik	11.788	11.095	1.617
Raet1a	10.889	10.196	1.617
Eef1e1	13.741	13.050	1.615
129828_f_at	13.058	12.367	1.615
1500009M05Rik	12.181	11.489	1.615
Wdr3	11.458	10.768	1.614
2010012G17Rik	10.301	9.612	1.612
Hmga2	11.394	10.705	1.612
2610044O15Rik	10.029	9.340	1.612
Kpna4	12.265	11.577	1.611
2700088M22Rik	12.280	11.592	1.611
Nup62	12.692	12.004	1.611
Nr4a1	10.317	9.630	1.610
Hiat1	11.221	10.534	1.610
Acat2	10.013	9.327	1.609
Exosc2	11.374	10.690	1.607
Actl6a	11.320	10.636	1.606
Mrpl53	10.640	9.957	1.606
165216_r_at	10.364	9.681	1.606
137581_f_at	12.975	12.292	1.605
Rnpc1	10.559	9.878	1.603
1110038G02Rik	11.286	10.606	1.602
103204_r_at	10.299	9.621	1.601
102098_at	14.214	13.535	1.600
Ahcyl1	14.330	13.652	1.600
0610009B22Rik	10.070	9.392	1.600
Ssx2ip	9.643	8.966	1.600
Uchl3	13.551	12.874	1.599
Slc35b1	12.470	11.793	1.598
Prps1	12.668	11.992	1.598
Ldh1	15.766	15.089	1.598
1500026D16Rik	11.881	11.205	1.597
Eno3	12.988	12.314	1.597
6530411B15Rik	11.302	10.628	1.595
9030611O19Rik	9.951	9.279	1.594
Tnpo1	12.584	11.912	1.594

163197_i_at	9.638	8.966	1.593
Hoxa9	11.411	10.739	1.593
Mast2	11.498	10.826	1.593
2310024N18Rik	11.856	11.185	1.592
3732409C05Rik	11.240	10.569	1.592
98077_at	12.471	11.800	1.592
Grcc3f	10.586	9.915	1.591
2010107E04Rik	13.013	12.343	1.591
Rbm14	10.899	10.230	1.590
Camk2d	11.840	11.172	1.590
168859_at	12.312	11.643	1.590
1110007M04Rik	11.023	10.355	1.589
Ahcy	13.130	12.463	1.588
4921513D23Rik	12.114	11.447	1.588
Pdk3	12.462	11.796	1.587
Ube3a	10.347	9.681	1.586
Mrps25	10.625	9.959	1.586
2610002D18Rik	11.804	11.139	1.586
166804_f_at	12.060	11.396	1.585
Slc9a3r1	12.038	11.374	1.584
Thrap2	11.087	10.424	1.583
1810023B24Rik	13.364	12.702	1.581
Mgst3	11.966	11.305	1.581
Nif3l1	11.325	10.665	1.581
Hint3	10.778	10.118	1.580
Smc6l1	11.951	11.291	1.580
Cbx3	11.032	10.372	1.579
Cdca1	11.672	11.012	1.579
Bri3bp	12.743	12.084	1.579
Dnajd1	9.769	9.111	1.578
Mfn2	12.482	11.824	1.578
3110050N22Rik	11.003	10.345	1.578
Rab22a	11.662	11.004	1.578
Prrx2	11.419	10.761	1.578
Srpx2	12.973	12.316	1.578
2610528M18Rik	9.627	8.971	1.576
Lmnb1	11.095	10.439	1.576
Wbscr18	12.130	11.474	1.576
102400_at	10.159	9.503	1.575
G630055P03Rik	11.132	10.477	1.575
Fgfrl1	12.790	12.135	1.575
S100a1	10.684	10.029	1.574
168172_r_at	10.679	10.024	1.574
1810020G14Rik	10.796	10.141	1.574

Trim59	11.428	10.774	1.573
110745_i_at	11.549	10.896	1.573
Rpl12	11.516	10.863	1.572
111998_at	11.370	10.718	1.571
166218_at	11.685	11.033	1.571
Erh	14.012	13.361	1.570
Usp1	11.879	11.229	1.570
Psmd8	12.321	11.671	1.569
C330027G06Rik	9.707	9.057	1.569
BC037006	9.989	9.340	1.568
Pdcd5	13.030	12.380	1.568
Tbl3	12.191	11.541	1.568
3732413I11Rik	10.914	10.265	1.568
Skp2	10.310	9.661	1.568
Ak5	11.072	10.423	1.568
Tial1	11.524	10.875	1.568
Nbea	11.023	10.375	1.567
Mpp1	13.156	12.509	1.566
Dr1	11.034	10.386	1.566
Pdk1	11.019	10.372	1.566
Drctnnb1a	12.757	12.111	1.565
Rnf128	11.691	11.045	1.565
105552_at	10.273	9.627	1.564
Ythdf3	12.147	11.502	1.564
163863_at	10.638	9.994	1.563
Hdgf	14.549	13.905	1.563
3300001G02Rik	11.871	11.227	1.562
Cycs	10.583	9.939	1.562
Mpdz	11.837	11.194	1.561
Wdr31	10.011	9.369	1.561
Nsdhl	11.070	10.428	1.560
Ppp1r14b	14.014	13.373	1.560
Ube2m	12.462	11.822	1.558
Prkca	9.722	9.083	1.558
1110061A14Rik	11.678	11.039	1.558
Dph2l1	10.726	10.087	1.558
1110008F13Rik	11.926	11.287	1.557
Arl7	11.221	10.582	1.557
106656_at	9.888	9.249	1.557
Pfn2	9.603	8.966	1.556
Uble1b	12.780	12.143	1.555
Etf1	13.075	12.439	1.554
Capza1	13.007	12.372	1.553
100880_at	10.155	9.520	1.552

Mapkapk2	10.541	9.907	1.552
BC033915	12.206	11.573	1.551
Pkn2	12.010	11.377	1.551
92615_at	13.322	12.689	1.550
Gap43	9.782	9.150	1.550
Kai1	10.961	10.328	1.550
2810003C17Rik	11.665	11.032	1.550
Coq7	9.621	8.988	1.550
134292_at	10.514	9.882	1.549
Tardbp	11.780	11.149	1.549
Mrpl18	12.033	11.402	1.549
Zfp422	10.207	9.576	1.549
Impa1	10.693	10.062	1.548
Ccnt1	11.017	10.386	1.548
Sumf2	10.444	9.814	1.548
Top2a	11.577	10.948	1.547
Med6	11.924	11.296	1.546
Park7	13.263	12.634	1.546
Cox7a2	14.057	13.428	1.546
Me2	10.892	10.264	1.545
Ddx51	9.593	8.966	1.545
2610510J17Rik	10.173	9.546	1.545
Uble1a	11.698	11.071	1.544
162900_at	12.762	12.135	1.544
Wsb2	12.353	11.728	1.543
Man1b	11.948	11.323	1.543
Tfrc	13.380	12.754	1.543
1110008B24Rik	11.618	10.993	1.543
110546_at	10.596	9.971	1.543
Hcfc1r1	13.867	13.242	1.543
Zmym1	10.344	9.719	1.542
Erf	11.277	10.652	1.542
Reps1	10.719	10.095	1.541
BC052066	11.539	10.915	1.541
Mrps6	13.704	13.080	1.541
Myo10	10.749	10.126	1.540
Etohi2	11.506	10.884	1.540
Dap	13.896	13.275	1.538
Camkk2	12.028	11.407	1.538
Metrn	13.714	13.094	1.538
Vbp1	13.664	13.043	1.538
4921517B04Rik	11.681	11.062	1.537
Glrb	9.585	8.966	1.536
Pogz	11.659	11.040	1.536

1110038B12Rik	14.477	13.858	1.536
2810025M15Rik	11.623	11.004	1.535
Usp24	10.881	10.263	1.535
2810052M02Rik	11.082	10.464	1.534
Oprs1	12.281	11.663	1.534
Cenpe	12.272	11.656	1.533
Echdc1	12.055	11.439	1.532
D7Wsu128e	11.336	10.720	1.532
114864_at	11.988	11.372	1.532
0610009E20Rik	11.464	10.850	1.531
A330080J22Rik	12.404	11.790	1.531
C330027G06Rik	10.445	9.831	1.531
Krt1-19	9.580	8.966	1.530
1110012J17Rik	11.821	11.207	1.530
Txndc7	13.053	12.440	1.530
Uqcr	12.498	11.885	1.529
Mrps28	10.749	10.136	1.529
Ubqln2	11.749	11.136	1.529
E330008O22Rik	9.761	9.149	1.529
2810036L13Rik	12.355	11.743	1.528
Akt1	12.934	12.323	1.528
Mdh2	14.351	13.740	1.527
1110057H19Rik	12.875	12.265	1.526
Arl7	11.878	11.269	1.526
Slc19a2	12.124	11.515	1.525
1300001I01Rik	11.737	11.129	1.524
Gak	12.099	11.492	1.524
Coq3	10.865	10.257	1.524
4931405B09Rik	10.904	10.297	1.523
Mcm2	12.039	11.431	1.523
1110034A24Rik	11.646	11.039	1.523
Gstm5	10.935	10.330	1.521
161872_f_at	9.586	8.982	1.521
4933403F05Rik	11.284	10.680	1.520
Qk	10.052	9.448	1.520
160416_at	10.736	10.132	1.519
Acp1	11.059	10.456	1.519
2310061I04Rik	12.020	11.418	1.519
129441_at	9.945	9.342	1.519
Rnf14	10.448	9.846	1.518
Fxyd5	11.338	10.737	1.517
Sypl	12.386	11.786	1.516
3110040D16Rik	11.829	11.229	1.516
1810021J13Rik	10.292	9.692	1.516

107895_at	10.565	9.965	1.515
Hdac3	10.953	10.354	1.514
Mybl2	10.036	9.437	1.514
163889_at	11.721	11.122	1.514
163900_at	12.035	11.437	1.514
2610529H08Rik	11.809	11.212	1.512
5730592L21Rik	11.786	11.189	1.512
2310016C16Rik	13.743	13.147	1.512
Rad51	10.930	10.334	1.511
2610019N13Rik	12.776	12.181	1.511
9030406N13Rik	11.138	10.544	1.510
Etv4	9.827	9.233	1.510
Tm4sf6	11.499	10.905	1.509
Txndc7	12.558	11.964	1.509
Pigk	12.045	11.452	1.509
Sap30	10.186	9.593	1.508
Pgam1	14.619	14.028	1.507
Myef2	10.944	10.352	1.507
2610012O22Rik	12.260	11.669	1.506
Mlr2	9.808	9.217	1.506
Psmd4	13.451	12.861	1.506
Acyp1	11.140	10.551	1.505
Nrd1	12.126	11.537	1.505
9930116P15Rik	11.118	10.528	1.505
Cdc42bpb	12.145	11.556	1.504
C630002M10Rik	9.555	8.966	1.504
A730024F05Rik	9.962	9.374	1.503
Fip1l1	10.243	9.655	1.503
Aldh1a1	9.553	8.966	1.502
Mrps22	10.507	9.921	1.501
Scrib	13.188	12.603	1.501
Grcc2f	13.227	12.641	1.501
Ppp2r5e	11.320	10.735	1.500
Increased in CD34-			
Nov	8.970	12.062	8.531
Fos	8.966	12.035	8.391
Enpp2	9.705	12.608	7.479
Itgbl1	9.258	12.020	6.783
114665_at	9.423	12.038	6.125
Pltp	10.098	12.648	5.856
Wisp2	9.209	11.598	5.239
Ctsk	8.966	11.150	4.545
Adamts2	10.987	13.008	4.056
96038_at	9.148	11.053	3.745

Cd59a	9.190	11.085	3.720
Col3a1	12.538	14.432	3.715
Marcks	9.950	11.821	3.657
BC064033	11.181	13.046	3.643
C1s	10.824	12.625	3.484
Col3a1	8.966	10.759	3.465
Col3a1	10.197	11.986	3.456
C130099A20Rik	10.824	12.544	3.294
Slfn2	9.792	11.499	3.265
Cxcl12	10.792	12.455	3.168
Txnip	9.815	11.448	3.101
Cpne8	9.431	11.048	3.067
Il1rn	9.003	10.612	3.051
H2-L /// H2-D1	9.502	11.096	3.017
Ogn	10.482	12.060	2.985
Cd47	10.185	11.753	2.966
Dhrs3	9.290	10.848	2.943
Klf4	9.959	11.503	2.917
107959_at	10.095	11.597	2.833
115084_at	10.331	11.820	2.807
Gadd45a	11.009	12.493	2.799
Hp	10.534	12.007	2.776
103709_at	13.694	15.165	2.773
4833416E15Rik	9.308	10.768	2.751
93714_f_at	13.531	14.980	2.731
H2-Q1	12.718	14.154	2.705
Casp4	8.966	10.400	2.703
Cd47	11.228	12.652	2.683
H2-Q2	12.439	13.849	2.658
Matn2	10.234	11.640	2.650
Tagln	12.987	14.385	2.636
98579_at	11.287	12.671	2.609
Junb	10.535	11.906	2.587
Cxcl12	9.206	10.565	2.565
Itm2b	12.814	14.167	2.555
Serpinb9	9.494	10.830	2.526
D0H4S114	10.416	11.751	2.523
Tfg	11.000	12.319	2.496
8430401C09Rik	10.923	12.238	2.487
136210_at	9.532	10.845	2.485
Ctsb	8.966	10.275	2.479
Aasdhppt	9.089	10.382	2.450
135189_f_at	13.251	14.544	2.450
Timp3	12.575	13.868	2.450

Tcn2	10.026	11.316	2.447
Islr	9.783	11.071	2.442
Hist1h1c	11.179	12.464	2.437
Gpm6b	12.196	13.477	2.430
AI194318	10.191	11.469	2.425
Ogn	10.785	12.058	2.416
Rhob	12.607	13.869	2.398
Rin2	10.611	11.867	2.389
Tnfrsf11b	9.751	11.004	2.384
Ndrl	12.026	13.277	2.379
Gpatc1	14.230	15.480	2.378
140833_at	11.303	12.545	2.365
163063_i_at	10.167	11.406	2.360
Cln2	10.343	11.580	2.357
H2-L	13.803	15.036	2.350
104363_at	10.362	11.593	2.348
Cnn1	11.858	13.088	2.346
Atf3	9.995	11.220	2.338
Acta1	9.188	10.412	2.336
106175_at	10.045	11.268	2.334
App	13.245	14.464	2.328
2310015N21Rik	11.533	12.750	2.325
Tapbp	12.805	14.015	2.312
169459_i_at	12.459	13.664	2.306
Ctsb	9.695	10.891	2.291
AI607873	9.695	10.881	2.275
2310061N23Rik	9.964	11.149	2.274
Fbxw9	9.548	10.732	2.273
Grn	12.530	13.712	2.270
Slfn2	9.050	10.223	2.256
Col1a1	15.191	16.360	2.249
101995_at	12.920	14.086	2.244
Serpinf1	13.602	14.766	2.242
Postn	14.104	15.246	2.207
Hes1	10.456	11.598	2.206
Cast	9.797	10.938	2.206
Fstl1	11.907	13.045	2.200
Cygb	10.470	11.606	2.197
Grn	13.149	14.283	2.194
H2-Q7	13.020	14.153	2.194
Osmr	9.985	11.113	2.186
H2-K1	13.873	14.995	2.176
93956_at	11.895	13.014	2.172
AB023957	9.161	10.279	2.171

165119_f_at	11.126	12.243	2.169
Ndrl	12.296	13.409	2.163
Anxa8	8.973	10.084	2.160
Nqo3a2	10.606	11.711	2.151
Mmp11	10.380	11.480	2.144
Colec12	12.791	13.880	2.128
Cln2	10.376	11.463	2.124
Col6a1	11.246	12.333	2.124
Cygb	11.231	12.315	2.119
Dppa4	8.994	10.071	2.109
Maoa	11.835	12.906	2.100
Cxcl12	12.012	13.075	2.089
Fkbp7	10.365	11.428	2.089
Serpinb6b	10.074	11.124	2.071
Man2b1	10.371	11.418	2.066
Smbp	9.662	10.708	2.064
Nupr1	11.757	12.802	2.064
92830_s_at	10.377	11.421	2.062
Mmp14	12.903	13.946	2.061
AI595338	9.937	10.979	2.059
Fbn1	10.815	11.857	2.059
166175_f_at	12.980	14.019	2.055
Phyh	9.090	10.126	2.051
BC051628	13.090	14.123	2.046
Ctsb	14.153	15.181	2.040
Col6a2	11.676	12.702	2.036
C330005L02Rik	9.755	10.776	2.030
Pla1a	12.083	13.104	2.029
Gpm6b	9.044	10.064	2.027
Col6a1	13.074	14.093	2.027
Mx2	8.966	9.980	2.020
Htr4	9.347	10.359	2.018
Ifi44	12.356	13.368	2.016
1700054O13Rik	9.467	10.475	2.011
Gaa	12.384	13.392	2.011
Ifi35	10.444	11.445	2.001
2310034L04Rik	10.945	11.945	2.000
Ctla2a /// Ctla2b	10.289	11.288	2.000
Ccdc3	12.393	13.393	1.999
H2-Q8	10.012	11.001	1.985
Glce	10.922	11.911	1.985
Oas2	11.279	12.264	1.979
93472_at	11.472	12.452	1.972
Fzd1	12.361	13.337	1.967

94813_at	11.341	12.312	1.960
97173_f_at	11.041	12.011	1.959
Pdir	10.109	11.076	1.954
164077_at	10.308	11.275	1.954
Gpnmb	13.368	14.332	1.950
Phf11	12.178	13.140	1.948
Fosb	9.920	10.879	1.945
Waspip	9.798	10.757	1.944
Mrc2	11.734	12.693	1.943
Cx3cl1	10.303	11.261	1.943
2810474O19Rik	11.032	11.990	1.942
Col4a1	13.639	14.597	1.942
Fzd1	8.966	9.923	1.941
164751_f_at	10.486	11.433	1.928
Naglu	10.233	11.179	1.927
165171_r_at	10.179	11.124	1.925
113335_at	11.151	12.095	1.923
H2-Q1	11.483	12.427	1.923
Sfrs7	9.826	10.764	1.916
Copg	10.239	11.176	1.914
166934_s_at	11.063	11.999	1.914
AI481100	10.710	11.646	1.913
Col4a2	13.887	14.820	1.909
Cx3cl1	8.978	9.909	1.907
Aspn	9.135	10.063	1.903
Csad	10.344	11.272	1.903
Hip2	9.850	10.777	1.901
Ckb	10.746	11.663	1.888
110107_at	10.964	11.881	1.888
2610001E17Rik	10.199	11.113	1.885
Aebp1	11.767	12.681	1.885
Fhl1	12.541	13.454	1.884
Xdh	10.266	11.174	1.876
C1r	10.481	11.389	1.876
Mrc2	9.825	10.731	1.874
Mtap4	10.332	11.231	1.865
98465_f_at	11.850	12.749	1.865
Adarb1	9.078	9.976	1.864
Ypel3	10.683	11.581	1.862
5930418K15Rik	10.234	11.128	1.858
Snn	9.840	10.733	1.857
Slc7a2	9.208	10.100	1.856
Rbpms	9.634	10.525	1.854
1010001D01Rik	10.687	11.577	1.854

106475_at	11.038	11.926	1.850
Itih2	11.571	12.456	1.847
Usp18	11.266	12.150	1.846
1500005P14Rik	11.402	12.285	1.843
Ccnh	9.277	10.157	1.840
1200009O22Rik	9.713	10.593	1.840
131836_at	10.368	11.247	1.840
Scotin	13.082	13.960	1.837
162249_f_at	9.186	10.062	1.836
Dtx4	11.836	12.712	1.835
103869_at	10.005	10.879	1.834
6230421P05Rik	9.415	10.285	1.828
Ralgds	11.098	11.968	1.828
113495_at	10.263	11.133	1.827
2310075C12Rik	12.294	13.163	1.826
Trib3	10.325	11.190	1.821
171236_r_at	10.241	11.103	1.818
Il6st	11.391	12.252	1.817
Socs3	9.808	10.669	1.817
Prrx1	11.753	12.614	1.816
H2-D1	13.854	14.712	1.811
Naga	10.944	11.800	1.809
Sox4	11.410	12.264	1.808
Dpp7	10.788	11.641	1.806
Gulp1	10.548	11.397	1.801
BC014685	9.821	10.669	1.800
Runx2	9.525	10.372	1.799
Lgals8	9.925	10.772	1.798
Isgf3g	10.798	11.644	1.798
D030068L24Rik	11.856	12.701	1.797
Nagk	9.256	10.100	1.795
9030024J15Rik	8.966	9.808	1.793
Igh-4	10.025	10.866	1.792
Ccl7	11.819	12.659	1.789
Junb	11.432	12.271	1.789
Sncg	10.017	10.854	1.787
Btbd14a	9.093	9.928	1.784
Cln8	10.669	11.503	1.783
100560_at	10.634	11.467	1.781
5730420B22Rik	9.168	9.998	1.778
Mustn1	11.850	12.680	1.778
Gtpbp2	10.867	11.697	1.777
Rnf125	9.194	10.022	1.775
BC003281	11.888	12.715	1.775

Phldb2	10.355	11.181	1.773
BC025600	13.273	14.099	1.772
Thbs2	13.033	13.857	1.770
Cast	10.183	11.006	1.769
129195_at	10.397	11.218	1.766
C920025E04Rik /// H2-T23	9.134	9.952	1.762
Tapbp	11.623	12.440	1.762
AI451465	10.306	11.123	1.762
H2-T10	11.565	12.381	1.761
135130_at	10.201	11.017	1.761
Ank	11.809	12.625	1.761
Lamp2	12.036	12.849	1.757
C330050A14Rik	8.966	9.779	1.757
103697_at	9.225	10.034	1.752
Zfp521	9.228	10.037	1.752
170245_r_at	9.088	9.896	1.751
Dnm	9.708	10.513	1.748
Mass1	9.741	10.545	1.746
5330414D10Rik	10.339	11.143	1.745
Agrn	10.668	11.468	1.741
167578_at	13.192	13.990	1.739
Capn5	9.298	10.095	1.737
Cckbr	14.500	15.296	1.737
Ly6a	14.756	15.551	1.736
Mpv17	9.433	10.226	1.734
Ifi205	12.239	13.030	1.731
Gig1	9.156	9.947	1.730
Wdr40a	10.319	11.109	1.730
A030012M09Rik	10.332	11.122	1.730
135383_r_at	13.536	14.325	1.728
Nfatc1	9.391	10.179	1.727
167574_r_at	11.298	12.086	1.727
165165_r_at	11.173	11.961	1.727
Fzd6	11.131	11.918	1.726
Gns	11.189	11.975	1.725
Lamp2	12.673	13.460	1.725
AI481105	9.480	10.266	1.724
Lamp2	12.606	13.390	1.722
2310036D22Rik	12.104	12.886	1.720
Nedd4	8.968	9.750	1.720
Cyp1b1	11.845	12.627	1.718
Hexa	11.029	11.809	1.718
164231_at	8.966	9.746	1.717

2300002D11Rik	11.047	11.825	1.715
Nutf2	10.585	11.363	1.714
1810010O14Rik	11.836	12.613	1.713
2010106G01Rik	10.531	11.307	1.712
Ebf1	9.381	10.155	1.710
Pgcp	10.278	11.051	1.709
Jup	9.633	10.406	1.709
103211_at	10.521	11.291	1.705
H2-L /// H2-D1 /// H2-K1 /// H2-Q2 /// H2-Bl	11.674	12.443	1.705
Qscn6	9.842	10.611	1.704
Mbtd1	8.983	9.752	1.704
161563_r_at	13.861	14.628	1.702
168363_i_at	12.861	13.627	1.701
Mapk12	9.292	10.056	1.699
Plk2	12.489	13.254	1.698
Timp2	11.779	12.542	1.698
AW549877	9.052	9.815	1.697
Cblb	9.761	10.521	1.694
Fstl1	10.326	11.086	1.694
Nek6	9.970	10.730	1.693
Igsf3	9.484	10.242	1.691
Ier2	11.955	12.711	1.689
Mustn1	11.018	11.773	1.688
Tcra	10.152	10.907	1.688
4930553M18Rik	11.173	11.926	1.686
108240_at	9.221	9.974	1.686
162699_at	11.040	11.793	1.686
Ddx5	11.460	12.212	1.685
Klf3	11.340	12.092	1.684
114328_f_at	10.570	11.321	1.682
Cpe	14.122	14.871	1.680
Klf3	9.630	10.378	1.680
Stat1	10.096	10.844	1.680
Lox	12.755	13.502	1.679
Actb	12.223	12.968	1.677
Ikbkg	9.692	10.437	1.676
Sh3gl1	11.573	12.318	1.675
Fhl1	11.967	12.709	1.674
103202_at	11.038	11.780	1.672
Cklfsf3	9.001	9.740	1.670
B2m	15.216	15.956	1.669
B3gnt1	9.486	10.225	1.669

161284_r_at	11.732	12.471	1.669
Pycr1	12.129	12.868	1.669
Dock9	9.906	10.645	1.669
0610007C21Rik	10.526	11.263	1.667
Bst2	14.845	15.582	1.667
171231_i_at	12.085	12.822	1.666
Rhoe	12.239	12.975	1.665
D330050I23Rik	9.183	9.916	1.662
Dpys	9.072	9.803	1.660
Dnm	10.211	10.942	1.660
Maoa	13.020	13.750	1.658
Col16a1	11.098	11.828	1.658
Copz2	11.216	11.945	1.658
Igtp	9.814	10.542	1.657
109832_f_at	12.534	13.260	1.654
105086_at	10.122	10.847	1.653
Rassf6	10.185	10.906	1.649
105912_at	11.164	11.885	1.649
Fyn	10.333	11.054	1.648
Spg21	12.793	13.510	1.645
Zmat2	11.806	12.522	1.643
Prss11	12.443	13.157	1.640
165080_r_at	9.022	9.735	1.639
167409_f_at	9.655	10.368	1.638
2900024C23Rik	10.631	11.343	1.638
Spred1	11.261	11.970	1.635
Dhx40	9.579	10.288	1.635
Cdc42ep1	12.635	13.344	1.634
Tsc1	9.255	9.963	1.633
1110014L14Rik	9.616	10.322	1.632
Ppp6c	11.859	12.566	1.632
Zfp262	10.614	11.320	1.630
4833416E15Rik	8.977	9.682	1.630
Emilin1	11.439	12.143	1.630
Ctsd	13.284	13.987	1.628
Grina	13.689	14.392	1.628
Plcb1	12.387	13.086	1.623
Runx1	9.004	9.703	1.622
110648_at	10.143	10.841	1.622
Map1lc3b	12.281	12.978	1.621
A630007B06Rik	9.775	10.470	1.619
Mte1	12.465	13.158	1.616
Lamb2	10.319	11.010	1.615
2410003B16Rik	10.646	11.335	1.612

D2Bwg0891e	11.127	11.816	1.612
Bat5	11.970	12.659	1.612
Itm2c	12.154	12.843	1.611
Pcolce	14.200	14.888	1.610
Nbr1	10.601	11.287	1.609
5430439C17Rik	10.454	11.139	1.607
E430026E19Rik	9.257	9.941	1.607
Smad3	11.565	12.248	1.606
108241_at	11.173	11.856	1.605
Ak3l	9.851	10.534	1.604
Cth	9.825	10.507	1.604
Col1a2	15.437	16.118	1.603
96530_at	11.030	11.710	1.602
Pitpnc1	9.883	10.562	1.601
Ccl2	13.040	13.719	1.601
E130107N23Rik	9.889	10.568	1.600
D130038B21Rik	13.067	13.745	1.600
1110008L20Rik	14.043	14.721	1.599
Col5a1	11.449	12.127	1.599
9130002C22Rik	12.059	12.735	1.597
2810474O19Rik	12.276	12.951	1.597
109751_at	10.871	11.547	1.597
H2-T10	12.074	12.749	1.597
1700110N18Rik	11.346	12.022	1.597
Col4a2	13.251	13.926	1.596
2600003E23Rik	10.244	10.918	1.596
Hmox1	12.674	13.347	1.595
Snag1	10.028	10.700	1.594
109495_at	11.200	11.869	1.591
Kitl	10.040	10.708	1.589
1200013A08Rik	14.335	15.000	1.586
D030029J20Rik	9.477	10.142	1.585
Rbm9	11.507	12.171	1.584
Thra	11.247	11.909	1.583
Cpe	13.746	14.408	1.583
Thbs1	14.802	15.462	1.580
Psap	13.785	14.444	1.579
Pgcp	10.741	11.399	1.577
S100a16	12.835	13.492	1.577
Ifit1	11.878	12.536	1.577
Ppgb	11.775	12.433	1.577
Scamp2	10.022	10.679	1.577
6720451E15	9.294	9.951	1.577
Il6st	10.552	11.209	1.576

168266_r_at	14.745	15.401	1.576
109176_at	13.443	14.099	1.576
129071_at	11.176	11.830	1.574
Nedd4	13.513	14.166	1.573
166739_r_at	13.573	14.226	1.572
3110001H15Rik	9.463	10.116	1.572
D430026P16Rik	12.599	13.251	1.571
131014_at	13.890	14.541	1.570
Prkra	10.625	11.275	1.569
2610005L07Rik	10.792	11.442	1.569
Pip5k2a	9.290	9.940	1.568
Nfia	11.995	12.643	1.568
134748_at	11.040	11.689	1.568
Fliih	11.027	11.675	1.567
Pdgfrb	10.809	11.457	1.567
Tor3a	9.422	10.068	1.565
112676_at	9.023	9.669	1.565
Centg2	11.936	12.581	1.564
Wdr13	9.676	10.319	1.562
Tgfb1i4	12.390	13.033	1.561
Nrbf2	10.507	11.149	1.561
Stat2	11.268	11.908	1.559
D8Ertd233e	9.820	10.461	1.559
163745_at	9.946	10.585	1.557
Slc7a5	13.155	13.793	1.557
97871_at	11.993	12.630	1.556
Ifrg15	10.734	11.370	1.554
Siat7b	9.005	9.641	1.554
Psmb10	11.306	11.941	1.554
Oasl2	12.908	13.543	1.553
1110021N07Rik	10.588	11.221	1.551
A930038C07Rik	10.301	10.934	1.551
Lims1	11.829	12.461	1.550
4732486I23Rik	10.062	10.694	1.549
Nav1	11.423	12.054	1.549
Axot	10.347	10.978	1.548
Colec12	12.546	13.177	1.548
Mglap	12.145	12.775	1.548
Mbnl2	13.646	14.275	1.547
Npdc1	11.382	12.011	1.546
Trim24	10.331	10.959	1.545
AI467484	9.736	10.363	1.544
Col6a3	14.271	14.897	1.544
2610200G18Rik	9.383	10.009	1.543

Hs6st2	9.182	9.808	1.543
Cast	11.342	11.966	1.542
Capn7	9.467	10.091	1.541
Smarca2	9.109	9.732	1.541
Peg13	11.532	12.155	1.540
BC027342	9.816	10.438	1.539
130720_at	10.257	10.879	1.539
Gnao1	10.287	10.909	1.539
Kng1	10.402	11.024	1.539
114327_i_at	10.588	11.209	1.538
Birc2	9.293	9.911	1.535
Gpha2	12.140	12.758	1.535
Atp6v1a1	11.378	11.995	1.534
Elf3	12.520	13.136	1.533
115397_at	11.634	12.250	1.533
Ube1dc1	10.406	11.021	1.532
1500001L15Rik	9.190	9.805	1.531
2610028F08Rik	9.352	9.966	1.530
Gga2	10.541	11.153	1.529
Trip12	9.043	9.655	1.528
108785_at	10.531	11.143	1.528
Siah1a	9.472	10.083	1.528
Nnt	11.221	11.831	1.526
Plekhb2	10.642	11.252	1.526
Fem1a	10.120	10.730	1.526
Sfrs6	11.198	11.807	1.526
AI448102	12.126	12.736	1.526
Arfrp2	10.732	11.342	1.526
Rab2b	9.659	10.268	1.526
2310067E08Rik	12.934	13.543	1.526
Nfil3	9.095	9.704	1.525
166655_at	10.514	11.121	1.523
Arhgef10	10.754	11.360	1.522
Rtn3	11.892	12.498	1.522
4931426K16Rik	9.451	10.056	1.521
Sardh	9.902	10.507	1.521
Stat1	11.786	12.389	1.519
Tnfrsf1b	10.177	10.779	1.519
Capn3	9.961	10.564	1.519
1110035L05Rik	9.693	10.296	1.519
Bmp1	10.307	10.908	1.517
4632413K17Rik	11.192	11.794	1.517
Renbp	11.794	12.395	1.517
Rab6b	10.287	10.888	1.517

Arid2	9.828	10.427	1.515
2810024B22Rik	11.068	11.667	1.515
2410018G23Rik	11.214	11.812	1.514
1110003E01Rik	11.725	12.323	1.513
Ptch1	11.725	12.322	1.512
2310014D11Rik	11.993	12.590	1.512
Atm	9.430	10.026	1.511
Cd97	9.242	9.837	1.511
2810439M11Rik	12.695	13.290	1.511
Ddit3	11.179	11.774	1.510
1810058I24Rik	10.601	11.196	1.510
93439_f_at	10.439	11.033	1.510
109439_at	9.203	9.797	1.509
Abhd4	12.463	13.056	1.508
Mgst1	8.966	9.556	1.506
Plekhc1	11.371	11.960	1.504
D8Ertd354e	10.235	10.824	1.504
4732481H14Rik	13.927	14.515	1.503
169553_r_at	10.247	10.836	1.503
2410003B16Rik	13.221	13.809	1.503
Tnip1	10.861	11.447	1.502
Slc24a3	11.085	11.671	1.501
Mgea5	9.959	10.545	1.501
Stag2	9.292	9.877	1.500

Appendix 2

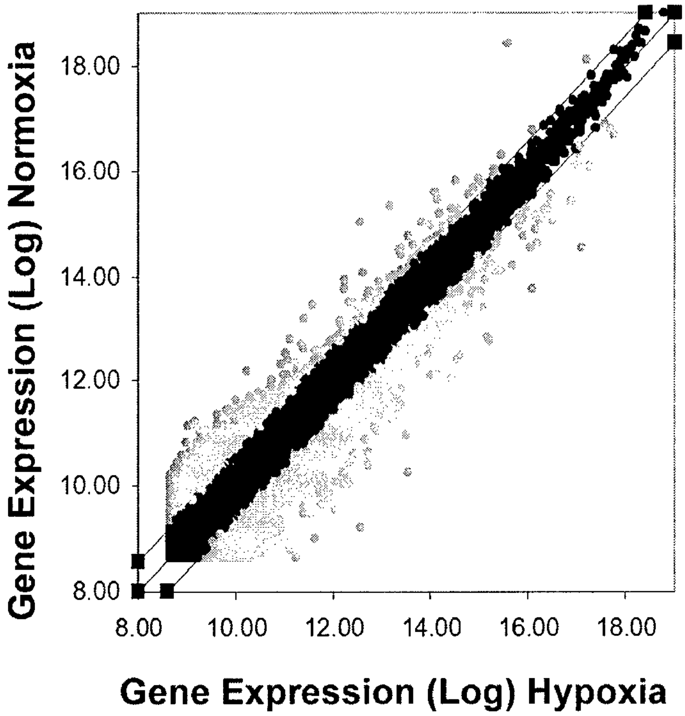

Figure 1: Scatter plot of differentially regulated genes between adult marrow derived stromal cells exposed to 24 hours of normoxia (20% O_2) or hypoxia (3% O_2). Light grey spots indicate genes which are altered by a least 2.5 fold between the groups.

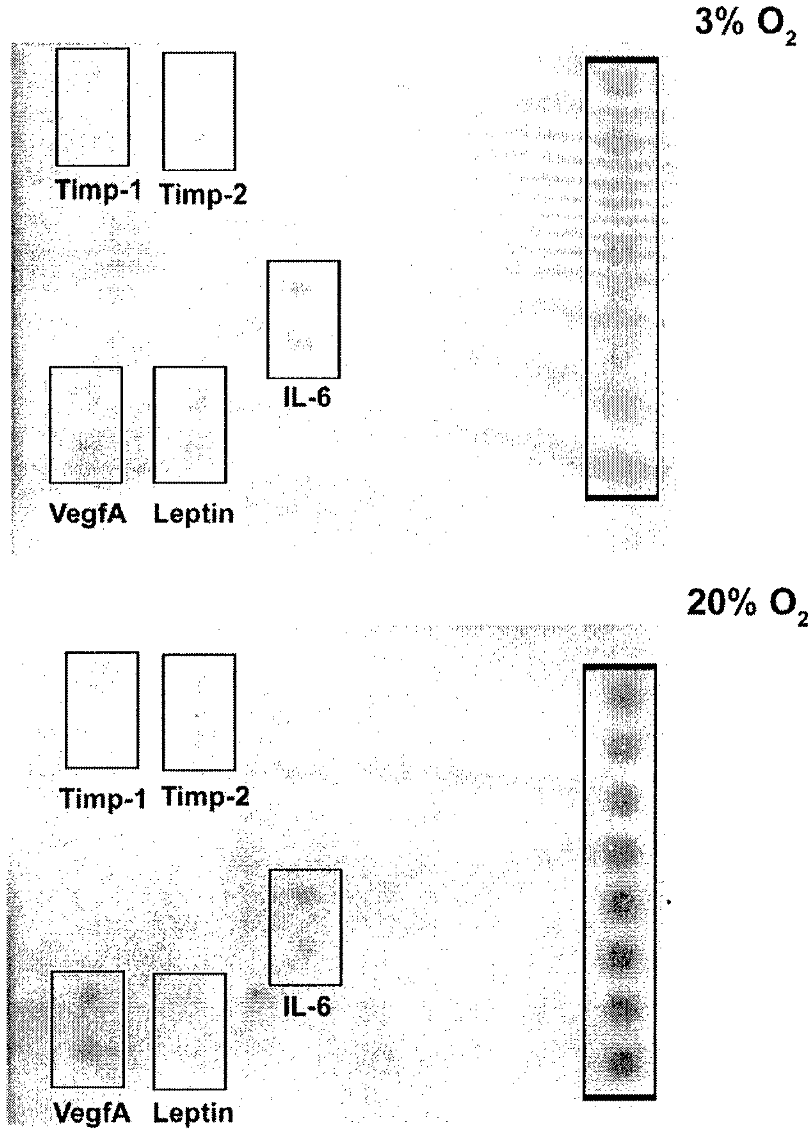

Figure 2: Panomics angiogenesis arrays measuring protein expression from the secretome of adult MSCs exposed to 24 hours of normoxia (20% O_2) or hypoxia (3% O_2). Exposure of adult MSCs to hypoxia cause a dramatic increase in the expression/release of Leptin.

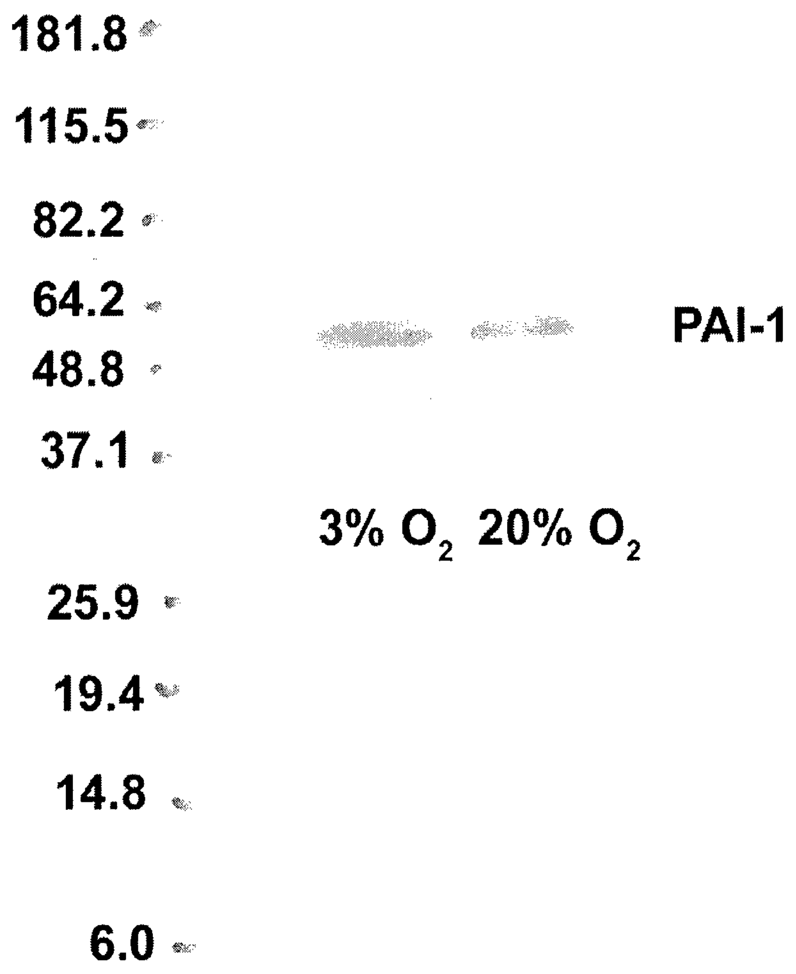

Figure 3: Western blot confirmation of ITRAQ mass spectrometry identification of plasminogen activator inhibitor-1 (PAI-1) as a protein whose expression/released from adult MSCs is increased due 24 hours exposure to hypoxia (3% O_2).

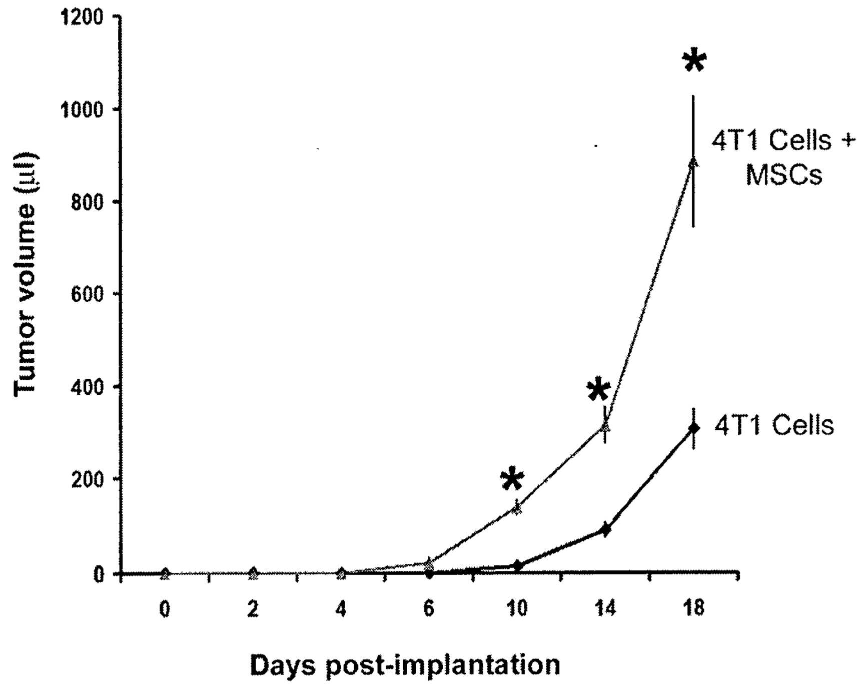

Figure 4: In vivo 4T1 tumor growth alone or in combination with adult MSCs. 10 days post-implantation there is a significant increase in tumor volume when 4T1 cells are implanted with adult MSCs. By 14 and 18 days this observations continues. (*; P<0.05).

Appendix 2

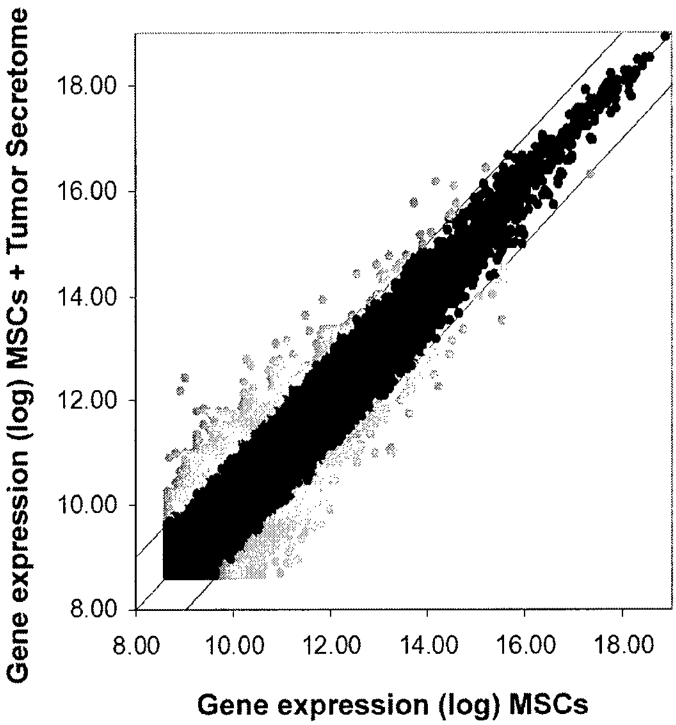

Figure 5: Scatter plot of differentially regulated genes between adult marrow derived stromal cells cultured alone or in the presence (contact independent) of 4T1 tumor cells. Light grey spots indicate genes which are altered by a least 2.5 fold between the groups.